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MAGELLANIC CLOUDS, V. FIFTY ECLIPSING STARS

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Communicated November 5, 1952

In preceding numbers of this series we have dealt with intrinsic variables.[1] In this communication we shall report on the geometric variables of the Magellanic Clouds. Since the eclipsing variables of our galaxy are so widely distributed in galactic latitude as well as in absolute magnitude, the first stars of this type to be discovered in the regions of the Clouds were assumed to be superimposed galactic variables.[2] As their number increased, Cloud membership was indicated; later it was satisfactorily proved. Table 1 gives the name, period, magnitudes, distance from geometric center, and type for 27 eclipsing stars in the Small Cloud and for 23 in the Large. RW Doradus—a fairly bright star of the W Ursae Majoris type, with a period determined by Hertzsprung—and the supergiant S Doradus have been omitted. The former is undoubtedly a foreground star; the latter, with its many peculiarities, including a P-Cygni spectrum, is in no way comparable with the other blue (presumably early B-type) stars here under consideration.

1. Cloud Membership.—When we determine the number of galactic eclipsing stars per unit area, to a given apparent magnitude limit, in fields outside the Clouds but in the same galactic latitude, the probabilities of cloud membership can be estimated for the variables appearing in the areas covered by the Clouds. Table 2a gives for the work on the Small Cloud the average number of eclipsing stars found for a unit area of 12.5 square degrees, for each magnitude interval from 12 to 16. The means are based on the discoveries in six thoroughly investigated variable star fields, totaling 400 square degrees, with a mean galactic latitude $\pm 41°$. The table also contains comparable data for a total of 14 fields, totaling a thousand square degrees with latitudes between $\pm 30°$ and $\pm 90°$.

In the last column is the actual number of eclipsing stars found in the

TABLE 1

ECLIPSING STARS IN THE MAGELLANIC CLOUDS[a]

NAME	PERIOD	MAXIMUM	MINIMA		ABSOLUTE MAGNITUDE	DISTANCE	TYPE
Small Cloud							
HV 1340	2.45889	15.75	16.55	15.9	−1.55	1.6	β Lyrae
1346	66.93	15.64	[17.1	...	−1.66	1.7	Algol
1441	1.02307	15.15	15.85	15.7	−2.15	1.1	β Lyrae
1485	7.1561	16.5	17.1	...	−0.80	0.6	Algol
1597	4.19275	16.25	17.0	16.4	−1.05	0.8	β Lyrae
1620	3.62043	14.20	14.7	14.6	−3.10	0.3	β Lyrae
1669	3.1888	14.5	15.3	15.1	−2.80	0.2	β Lyrae
1761	1.471116	15.35	16.0	15.9	−1.95	0.6	β Lyrae
1876	2.757997	15.05	15.7	15.5	−2.25	0.5	β Lyrae
1962	8.78596	14.84	15.54	15.10	−2.46	0.7	β Lyrae
1998	0.8831956	14.7	15.3	15.3	+5.0	0.9	W Ursa Maj
2016	2.95446	14.35	15.3	14.8	−2.95	0.8	β Lyrae
2092	1.77155	15.64	16.34	16.04	−1.66	1.2	Algol
2208	3.21277	14.9	15.8	15.2	−2.40	1.3	β Lyrae
2220	4.37905	14.65	15.4	14.95	−2.65	1.7	β Lyrae
2226	1.89983	15.44	16.26	15.86	−1.86	2.0	β Lyrae
11263	23.535	15.6	16.15	16.10	−1.70	0.8	β Lyrae
11284	3.62549	14.85	15.6	15.3	−2.45	0.6	β Lyrae
11301	1.27665	16.1	17.0	16.7	−1.20	0.8	β Lyrae
11308	3.2868:	15.95	16.5	16.2	−1.35	0.7	β Lyrae
11315	2.00335	15.0	15.8	15.6	−2.30	0.6	...
11321	2.08597	15.85	16.7	16.05	−1.45	0.5	β Lyrae
11345	1.176027	15.5	16.6	15.7:	−1.80	0.2	Algol?
11379	1.312793	16.4	17.0	16.7	−0.90	0.2	β Lyrae
12102	1.04318	16.2	16.8	16.5	−1.10	0.3	β Lyrae
CV 9	0.83629	16.4	17.0	16.7	−0.90	0.3	β Lyrae
"n" Columbia[b]	4.6068	14.0	15.0	14.75	−3.30	0.6	...
Large Cloud							
HV 936	10.0316	14.55	15.85	14.68	−2.75	1.3	Algol
940	3.01988	14.9	16.1	15.25	−2.40	0.6	Algol?
1022	2.5221	15.45	16.04	15.58	−1.85	2.2	β Lyrae
2241	4.34263	12.55	13.52	13.2	−4.75	3.1	β Lyrae
2274	5.7261	13.75	14.60	14.5	−3.55	1.9	β Lyrae
2329	4.1764	15.3	16.2	15.5	−2.00	1.6	β Lyrae
2348	1.99001	13.85	14.85	14.45	−3.45	1.5	β Lyrae
2425	5.8686217	15.70	16.60	16.03	−1.6	0.5	β Lyrae
2436	3.01115	15.75	16.60	15.90	−1.55	1.2	β Lyrae
2691	1.2555	15.25	15.8	15.5	−2.05	1.2	Algol?
2744	...	15.2	15.7	...	−2.1	1.7	...
2774	3.65242	15.1	15.7	15.3	−2.2	1.6	β Lyrae

TABLE 1 (Continued)

ECLIPSING STARS IN THE MAGELLANIC CLOUDS[a]

NAME	PERIOD	MAXIMUM	MINIMA		ABSOLUTE MAGNITUDE	DISTANCE	TYPE
5622	...	14.5	15.1	...	−2.8	1.4	...
5837	3.41755	15.75	16.4	15.9	−1.55	0.7	β Lyrae
5846	1.715033	15.6	16.5	16.1	−1.7	0.6	β Lyrae
5989	1.3069	15.25	15.75	15.65	−2.05	1.9	β Lyrae
11981	1.511	16.3	17.0	...	−1.0	1.3	...
11999	2.008036	14.7	16.7	...	−2.6	1.2	Algol
12071	7.283745	14.8	15.8	...	−2.5	2.9	...
12072	...	15.7	16.4	...	−1.6	2.7	...
12190	7.18282	14.0	15.05	...	−3.3	3.0	Algol
12228	...	16.2	16.9	...	−1.1	1.1	...
12244	...	12.9	14.0	...	−4.4	4.1	...

[a] The positions of these variables can be found through the following references: *Harvard Bulletins* 919 (1949), 921 (1952) and *Harvard Reprint* Ser. II-36 (1951).

[b] Variable found by Miss H. H. Swope on plates taken by Yale for Columbia. Position in the coordinates of *Harvard Annals* **60**, no. 4 (1907): $X = 11719''$, $Y = 8655''$.

TABLE 2

COMPARISON OF AVERAGE NUMBER OF GALACTIC ECLIPSING STARS[a] TO CLOUD NUMBER

(*a*) Small Cloud (Average Number of Eclipsing Stars in 12.5 Square Degrees)

MAGNITUDE	USING 400 SQ. DEG. AROUND $\beta \pm 41°$	USING 1000 SQ. DEG. $\beta \pm 30°$ to $\beta \pm 90°$	FIELD OF SMALL CLOUD
]12	0.32	0.15	0
12–13	0.25	0.20	0
13–14	0.47	0.30	0
14–15	0.37	0.25	8
15–16	0.03	0.03	12
[16	0.00	0.00	6

(*b*) Large Cloud (Average Number of Eclipsing Stars in 25 Square Degrees)

MAGNITUDE	USING 400 SQ. DEG. AROUND $\beta \pm 41°$	USING 1000 SQ. DEG. $\beta \pm 30°$ to $\beta \pm 90°$	FIELD OF LARGE CLOUD
]12	0.63	0.30	0
12–13	0.50	0.40	2
13–14	0.94	0.60	2
14–15	0.75	0.50	6
15–16	0.06	0.07	11
[16	0.00	0.00	2

[a] For data on the variable star fields used in this table see these PROCEEDINGS, **37**, 140 (1951), and Walter J. Miller, S.J.: "Harvard Variable Star Fields," *Ricerche Astronomiche*, **I**, No. 11 (1946): *Harvard Reprint* 290 (1947).

Small Cloud up to the present time. The number of galactic eclipsing stars falls off markedly for magnitudes fainter than 14.5, where the eclipsing stars in the Small Cloud begin to appear. All the eclipsing stars in the Cloud field are presumably Small Cloud members. No superimposed eclipsing stars are expected fainter than magnitude 15.0 in latitude $-45°$.

For the work on the Large Cloud, table 2b gives similar material, except that the area for comparison has been enlarged to 25 square degrees. The two brightest eclipsing stars, which are also the two farthest from the geometrical center of the Cloud, are probably foreground stars. For them spectroscopic study will be necessary to determine their absolute magnitudes and Cloud membership. To the others Cloud membership can be assigned.

2. Absolute Magnitudes.—With the modulus* of the Clouds taken as $17^m.3$, the absolute magnitudes, assuming Cloud membership, is given in the sixth column of table 1. The absolute magnitudes for the Small Cloud are fainter than for the Large: means are $M = -1.91$ and $M = -2.38$ respectively. When the three brightest are excluded, the mean is -2.71 for the Large Cloud. The upper limit of $M = -3.5$ is in keeping with magnitudes found for galactic early B eclipsing stars. Hubble's eclipsing star in M 33 has $M = -3.3$,[3] and Baade's star in IC 1613 has $M = -2.6$.[4]

3. Periods.—The periods (second column of table 1) are distributed from 0.836 to 66 days, with the majority less than three days. HV 1998, a W Ursae Majoris star, with period $0^d.8831956$ and range 14.7 to 15.3, is about seven magnitudes too bright for Cloud membership, if all the widely distributed W Ursae Majoris stars are alike and have absolute magnitudes near $+5.0$. From the list of five eclipsing stars published by Dr. M. Dartayet and J. L. Dessey of Córdoba[5] only two are included in this list: CV 229 = HV 12102 and CV 9. They are here given with magnitudes based on Harvard sequences to make the material more homogeneous. CV 9 is affected by overlapping images on plates made with the 24-inch refractor. The recorded range is undoubtedly too small.

Periods for all the known eclipsing variables in the Small Cloud have been determined, but in the Large Cloud only 18 of the 23 have yielded their periods as yet. There are no marked differences as to periods, ranges, and types between the Clouds, and no unusual phenomena such as rotating lines of apsides and eccentric orbits. The light curve of HV 11284 (fig. 1) is typical of the group: β-Lyrae rounded maxima, unequal minima, and partial eclipses. Five of the variables are algols with flat maxima, deep minima, and only slight indications of secondary minima. The periods are based on at least 100 observations covering five years or more. Because of photometric inexactitude, however, only half a dozen light curves are of such quality as to warrant preliminary discussion of orbits. All the stars

are too faint for easy photoelectric study with available equipment at Harvard's southern station.

4. Conclusion.—The distances from the centers of the Clouds (seventh column of table 1) reflect more the selection of the fields studied than the true distribution. Many eclipsing stars await discovery, particularly in the Large Cloud. In the Small Cloud to magnitude 16.5 we expect the number of undiscovered eclipsing stars to be not more than a dozen.

Comparing the number of eclipsing stars with the number of cepheids, we find in the Small Cloud a similar percentage (3%) for the dense inner body of the system, the border, and the outer edges. This similarity supports the hypothesis of Cloud membership for the eclipsing stars.

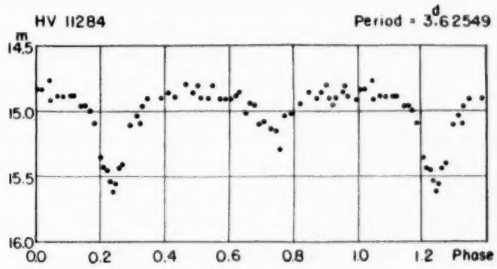

FIGURE 1

In the Large Cloud, however, the ratio of eclipsing stars to cepheids varies considerably from region to region. There seems to be a concentration near 30 Doradus.[6] The discovery of four eclipsing variables of approximately the same magnitude, 15.2, in one square degree about this bright nebulosity was our first indication that eclipsing stars were associated with the Cloud. As more regions are completely surveyed, more eclipsing stars should be found; it will be of interest to see if more are discovered in regions obviously affected by dust and gas than in some of the clearer regions of the Cloud. Such an affiliation would obviously bear on the theories of stellar evolution.

* A substantial revision of the zero-point of the period-luminosity relation will probably be made in the course of a year or so, and both the distances to the Clouds and these absolute magnitudes will be altered thereby. The luminosities and the distances, as well as Cloud dimensions, will be much increased. The correction will not alter the relative magnitudes within the Clouds.

[1] These PROCEEDINGS, **37,** 138–145 (1951); *Harvard Reprint* 346 (1951).

[2] *Harv. Obs. Bull.* 916 (1942).

[3] Hubble, E., *Ap. J.*, **63,** 104 (1926); *Mt. Wilson Contribution* 310, 22 (1926).

[4] Annual Report of the Director of the Mount Wilson Observatory, p. 19 (1942); Carnegie Institution Year Book, **41,** 19 (1942).

[5] Dartayet, M., and J. L. Dessey, *Ap. J.*, **115,** 279–283 (1952).

[6] THESE PROCEEDINGS, **34,** 177–178 (1948); *Harvard Reprint* 306 (1948).

EVIDENCE FOR THE SYNTHESIS OF VITAMIN B_{12} BY SPONTANEOUS TUMORS*

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Communicated November 7, 1952

Many attempts have been made to discover a metabolic difference[1] between corresponding normal and neoplastic tissues of an individual. The reasons for such efforts are clear. If a specific metabolic reaction could be found to take place in malignant tissues, and not to occur in the corresponding normal structures, a definite point of departure would be available for a more precise study of the nature of the disease, and also possibly for the control of it. A qualitative metabolic difference, if it were found to occur, would make possible a more concise biochemical definition of the neoplastic tissue, but even a quantitative difference in the extent of some isolated reaction, if it were to be established securely, might prove to be a considerable advance in knowledge, even though the qualitative difference would probably be the most useful. Therefore any evidence which would indicate a difference in some specific metabolic reaction, or connected set of reactions, is noteworthy.

In previous papers[2-5] it has been shown that selectively acting antimetabolites can be realized which have a predictable ability to harm one kind of living thing, and to leave others untouched. This selectivity has been viewed as a prime requisite of successful chemotherapeutic agents. The preceding work has indicated that one of the ways in which such discriminatory action can be achieved is to choose a precursor of metabolites concerned in the intimate processes of cell division, and to make structural analogs of this precursor. Such antimetabolites will then damage those kinds of cells which conduct the synthesis of the vitamins (or other vital metabolites) from the aforementioned precursor, but will not touch those cells which do not. These latter kinds of cells can be distinguished readily from the former because they show their inability to conduct the synthesis of the vitamins by requiring them as essential constituents of their food. Thus, by knowing the dietary requirements of living things for certain compounds important in processes of cell division, and by knowing the precursors from which these are made in some organisms, it is possible to construct antimetabolites with a predictable selective action.

In order to achieve selectively acting agents which will function in a mixed population, a further refinement must be made.[4, 5] This is to choose a precursor which is common to the synthesis of two or more of the vitamins. In the cases examined this seemed necessary as one way to avoid the nullification of the antimetabolite effect by the vitamins themselves.

The particular example most studied involved riboflavin and vitamin B_{12} as the vitamins, and dimethyldiaminobenzene as the common precursor of them. Analogs of this precursor, such as dichlorodiaminobenzene and several others,[6] inhibited the growth of those micro-organisms and higher forms which manufactured their own riboflavin and vitamin B_{12}, but left unharmed those species which showed a dietary need for these vitamins. Higher animals, such as mice, were among the vitamin-requiring species, and they were not harmed detectably by the antimetabolites.

If, now, the neoplasms which affect mammals were to be found capable of synthesizing their own supplies of riboflavin and of vitamin B_{12}, one would expect that these tissues, like other living things so constituted with respect to these vitamins, would be susceptible to the action of these antimetabolites, whereas the animal as a whole would not be. A clear basis for chemotherapeutic attack would thus emerge.[7] The purpose of this paper is to present evidence that the spontaneous mammary cancers of mice synthesize vitamin B_{12}.

The proper way to determine the nutritional needs of cancers would be to grow them by themselves, free of normal tissue, in a culture fluid of known chemical constitution. At the present time this is technically impossible. The demonstration of vitamin B_{12} synthesis was therefore dependent on the taking advantage of the tenacious storage of this vitamin by animals. It is now well known that parents who consume an adequate diet pass on to their offspring enough vitamin B_{12} to meet almost completely the dietary needs of the young for normal growth, even when these progeny are fed a B_{12}-deficient ration.[8, 9] On the other hand, when mothers are maintained on the deficient diet during gestation, and the young are similarly fed, signs of avitaminosis are seen in these young. Presumably, the stores within the maternal organism are sufficiently reduced by this process to allow demonstration of the essential character of this vitamin in the offspring.[10]

Consequently, if a tumor-bearing mother were carried through gestation and nursing on a vitamin B_{12}-deficient ration, and if the neoplasm were synthesizing this vitamin in considerable amounts, the young would be adequately fortified, and would grow normally when continued on the deficient diet. The tenacious retention of vitamin B_{12} might thus allow us to study the capacity for synthesis possessed by the cancer growing in the mother.

EXPERIMENTAL

Rations.—The B_{12}-deficient ration, number PR 60, of Zucker and Zucker[11] was used. The principal component of this ration was cottonseed meal. Whenever it was desired to make this diet nutritionally adequate, 10 γ of crystalline vitamine B_{12} was added per 100 g. This was

considerably more than the amount required for the maximal response of growing mice.

Behavior of Normal Swiss Mice on the Deficient Ration.—Although the following preliminary experiments contributed no new knowledge about vitamin B_{12}-deficiency, they are recorded in order to show that under the conditions here employed, these basic facts could be demonstrated. The need for so doing will be quite clear in the remainder of the paper.

Normal females of the Rockefeller Institute Swiss mice in groups of 4 were fed the deficient ration for about a week, and were then introduced into a cage containing a male which was similarly fed. The cages were changed weekly, or more often[12] if they became fouled with food, in order to avoid contamination with vitamin B_{12} from microbial growth. As the females approached parturition, they were caged separately on shavings. The number of young in each litter was reduced to 6 after the first few days.

When the young were 21 days old they were weighed, and caged individually in metal boxes with screen floors. The deficient ration was continued. At the time of weaning, the B_{12}-deficient animals usually weighed, on the average, 9 g. The variation among individuals in a single litter and between litters, was not greater than 1.5 g. In other words, the starting weights, at weaning time, ranged from 8.0 to 10.5 g. The rate of growth was determined for a period of 2 weeks. At this time the animals in the supplemented groups were approaching maturity, so that a longer period of observation was impractical.

Because the difference in rate of growth between deficient and supplemented groups in any experiment with vitamin B_{12} is small, several repetitions were made to determine the reproducibility of the results, and the numbers of animals needed for statistical assurance. The data shown in table 1 indicate that the average gain in weight of groups of mice treated in the manner described was reproducible and precise enough for the purpose in hand. This demonstration was considered to be of great importance to the interpretation of the results of this study. Further data to illustrate the magnitude of individual differences will be found in table 5.

Demonstration of Storage of Vitamin B_{12} by the Mothers.—Mice were handled as described in the preceding section, except that the parents were supplied the supplemented ration (10 γ vitamin B_{12} per 100 g. of ration) up to the time of parturition. They were then given the deficient ration, and, following weaning, the growth rate of the young was determined while they were being fed the deficient diet. From the data of table 2 it is clear that such young mice grew at a rate only slightly less than that of those which were constantly supplied with more than adequate amounts of the vitamin. During gestation, it was concluded, enough vitamin B_{12} was stored by the mother, and passed on to the embryonic young, to suffice for the rearing of these young.

Time Required to Deplete the Stores of Vitamin B_{12}.—At various times during the gestational period, pregnant mice were removed from adequate stock rations and placed on the deficient diet. They were continued on this deficient regime, and the growth rate of the young, fed the same ration after

TABLE 1

VARIATION IN RESPONSE OF YOUNG GROWING MICE FED THE B_{12}-DEFICIENT RATION

DATE OF EXPERIMENT	NO. OF ANIMALS	AVERAGE GAIN, G. PER WEEK
Oct., 1951	10	3.4
Feb., 1952	11	3.3
June, 1952	30	3.1

TABLE 2

GROWTH RATE OF YOUNG MICE FED THE VITAMIN B_{12}-DEFICIENT RATION, BUT FROM MOTHERS FED THE VITAMIN

DIET FED YOUNG	NO. OF ANIMALS	AVERAGE GAIN IN WEIGHT, G. PER WEEK
B_{12}-deficient	6	5.0
B_{12}-deficient	10	4.9
$+B_{12}$	19	5.1

TABLE 3

TIME REQUIRED TO DEPLETE THE STORES OF VITAMIN B_{12} IN MOUSE MOTHERS AS MEASURED BY THE GROWTH RATE OF THEIR LITTERS FED THE DEFICIENT DIET

TIME BEFORE PARTURITION WHEN THE FEEDING OF THE DEFICIENT DIET WAS BEGUN, DAYS	NO. OF ANIMALS	AVERAGE GAIN IN WEIGHT, G. PER WEEK
3	5	4.2
14	16	4.0[a]
14	12	3.6[a]
21	16	3.4

[a] One of these groups was examined in May and the other in January. The remaining data were collected in January.

TABLE 4

GROWTH RATES OF WEANLING MICE FROM TUMOROUS MOTHERS COMPARED TO THOSE FROM NORMAL MOTHERS. BOTH MOTHERS AND YOUNG WERE FED THE VITAMIN B_{12}-DEFICIENT RATION EXCEPT WHERE NOTED

CONDITION OF MOTHERS	RATION FED YOUNG	NO. OF ANIMALS	AVERAGE GAIN IN WEIGHT, G. PER WEEK
Normal	B_{12}-deficient	62	3.3
Tumorous	B_{12}-deficient	43	4.4
Tumorous	$+B_{12}$	7	5.4
Normal	$+B_{12}$	19	5.1

weaning (21 days of age) was determined. The results in table 3 show that the stores of vitamin B_{12} were reduced to a minimum (as reflected by the growth rate of the young) when the mothers were started on the deficient ration at the time of mating (21 days before parturition). If the deficient

diet was begun any later than that, the young were subsequently found to grow faster, and this was probably due to the carrying over of some of the vitamin from the mother. Clearly then, prospective mothers must be started early on the deficient ration if the young from them are to be usable for the demonstration of vitamin B_{12}-deficiency.

Synthesis of Vitamin B_{12} by Spontaneous Mammary Cancers of Swiss Mice as Evidenced by Growth of Siblings on Deficient Rations.—Tumor-bearing mice were obtained from the colony of Swiss mice of our Institute. These spontaneous mammary cancers were particularly suited for the present work, because, although they were transplantable and otherwise gave clear evidence of malignancy[13] (e.g., they invariably killed their hosts), they progressed slowly. Thus, most mice carrying a tumor at least $1 \times 1 \times 0.5$ cm. would conceive, bear litter, and wean it before the tumor had become open and necrotic, and before the animal became moribund. This was of importance to the present experiments for obvious reasons. If one is to depend on the growth rate of young animals for one's conclusions, these young must come from mothers which are well enough to nurse a litter adequately. Furthermore, because some micro-organisms are known to synthesize vitamin B_{12}, the growth of such organisms in a necrotic tumor could seriously complicate the interpretation of results. Consequently, in the following experiments, animals from mothers which were exceedingly ill, or which had tumors that had opened, were avoided.[14] The taking of these precautions, and even more, the low fecundity of cancerous females, made the procurement of adequate numbers of animals a most difficult task.

Groups of 3 females, each bearing a spontaneous mammary tumor at least 1 cm. long, 1 cm. wide, and 0.5 cm. high, were caged with a normal male of proved fertility, and fed the deficient ration. The same precautions were observed as in the case of the first experiments (those with non-cancerous animals) described in this paper. Table 4 shows the growth rates of the weanlings from these tumorous mothers. It is evident that they grew considerably more rapidly than did those from non-tumorous mothers, and approached the gains of those from normal mothers which had received the B_{12}-supplemented diet during gestation (cf. table 2).

The greater rate of gain of mice from tumor-bearing mothers could have been due to an innate difference of these from the normals, without respect to vitamin B_{12}. This question was examined in the following way. A group of weanlings from tumorous mothers, which had been kept on the deficient diet, was fed the supplemented ration during the period when their growth-performance was measured. If there had been an innate constitutional difference of these from the normals which made them grow faster, then they should have gained more than did similarly supplemented weanlings from non-tumorous mothers. Actually, however, the two groups

gained weight at approximately the same rate (5.4 g. per week for the ones from tumorous mothers, and 5.1 g. per week for those from normal mothers). The slight difference was not considered significant.

Validity of the Major Conclusion.—The major conclusion, namely, that young mice from tumor-bearing mothers grew faster on a B_{12}-deficient regime than those from normal mothers, was borne out each of the four times the experiment was tried. Representative detailed data concerning individual mice are contained in table 5. This table was constructed by listing chronologically all of the mice in the entire study. All of the animals from normal B_{12}-deficient mothers were grouped together, as were also all from B_{12}-supplemented mothers, and all from tumorous, deficient mothers. In order to select the 12 individuals for each group shown in table 5, every fifth (or third as the case may be) mouse in the list was chosen. In this way a random selection of data was made. The values in table 5 will indicate the amount of variation in individual animals, and will also show representative starting weights of the young and the sizes of tumors in mothers. It is important to emphasize that because of this random selection of the data all of the individuals in any one experiment could not be shown in table 5. The only exception is in the case of young from mothers bearing transplanted tumors, where the data from all mice in 2 litters are recorded.

Evidence in Other Strains of Mice for Synthesis of Vitamin B_{12} by Spontaneous Mammary Tumors.—Because of the more rapid progression of spontaneous mammary cancers in the C3H and in C strains[15] over that seen in Swiss mice, the problems of procurement of adequate litters described above for the Swiss were intensified. Furthermore, tumorous females of the C3H and C strains were available to us in much smaller numbers. Only 2 experiments were possible with each of these strains fed the deficient ration, but in these the rate of gain of the young was such as to suggest that the same phenomenon as that observed with Swiss mice was discernible. The average gain per week of the young from C3H tumorous mothers was 4.3 g., and from C strain mothers was 4.1 g., compared to 3.5 g. for those from normal mothers similarly fed.

Failure of Transplanted Mammary Tumors of Swiss Mice to Give Evidence of Vitamin B_{12}-Synthesis.—Although spontaneous mammary cancers of Swiss mice gave clear evidence of synthesis of the vitamin, these same tumors transplanted into Swiss mice did not do so. On the day of mating 11 normal females were inoculated with small fragments of a spontaneous mammary tumor such as those carried by the animals previously described. These recipients were then fed the B_{12}-deficient ration. Five of the transplants began to grow, and were readily discernible at the time of parturition. The deficient diet was continued, and at weaning the growth rate of the young was determined as described previously. At the same time a

TABLE 5

RANDOMLY SELECTED RECORDS OF INDIVIDUAL MICE

CONDITION OF MOTHER	SIZE OF TUMOR AT WEANING, CM. × CM. × CM.	DIET FED MOTHER	DIET FED YOUNG AFTER WEANING	MOUSE NO.	STARTING WEIGHT OF YOUNG (21 DAYS OLD), G.	WEIGHT OF YOUNG AT 35 DAYS, G.	GAIN PER WEEK, G.	DATE OF EXPERIMENT
Normal	...	Deficient	Deficient	1-2	8.0	16.0	4.0	May, 1951
	...	Deficient	Deficient	6-1	8.0	14.0	3.0	May, 1951
	...	Deficient	Deficient	1	9.0	12.0	1.5	Oct., 1951
	...	Deficient	Deficient	6	8.0	17.5	4.8	Oct., 1951
	...	Deficient	Deficient	56	9.5	16.0	3.2	Feb., 1952
	...	Deficient	Deficient	61	9.5	16.0	3.2	Feb., 1952
	...	Deficient	Deficient	37	9.5	17.0	3.8	Feb., 1952
	...	Deficient	Deficient	110	9.5	16.0	3.2	June, 1952
	...	Deficient	Deficient	115	10.0	15.5	2.7	June, 1952
	...	Deficient	Deficient	120	9.0	15.0	3.0	June, 1952
	...	Deficient	Deficient	125	9.0	17.5	4.3	June, 1952
	...	Deficient	Deficient	130	9.0	17.0	4.0	June, 1952
	...	Plus B_{12}	Deficient	11	9.0	18.5	4.8	Oct., 1951
	...	Plus B_{12}	Deficient	12	9.5	20.0	5.3	Oct., 1951
	...	Plus B_{12}	Deficient	13	9.5	20.0	5.3	Oct., 1951
	...	Plus B_{12}	Deficient	14	10.0	18.5	4.3	Oct., 1951
	...	Plus B_{12}	Deficient	15	10.0	20.5	5.3	Oct., 1951
	...	Plus B_{12}	Deficient	16	10.0	17.5	3.8	Oct., 1951
	...	Plus B_{12}	Deficient	17	10.0	21.5	5.8	Oct., 1951
	...	Plus B_{12}	Deficient	18	10.0	19.5	4.8	Oct., 1951
	...	Plus B_{12}	Deficient	26A	12.0	22.5	5.3	Mar., 1951
	...	Plus B_{12}	Deficient	27A	10.0	19.0	4.5	Mar., 1951
	...	Plus B_{12}	Deficient	28A	8.0	17.0	4.5	Mar., 1951
	...	Plus B_{12}	Deficient	29A	8.0	20.0	6.0	Mar., 1951

Spontaneous mammary tumor	. . .	Deficient	Deficient	32	9.3	18.0	4.4	Oct., 1951
	. . .	Deficient	Deficient	34	10.0	18.5	4.3	Oct., 1951
	. . .	Deficient	Deficient	36	9.6	20.0	5.2	Oct., 1951
	3.0 × 2.8 × 1.8	Deficient	Deficient	62	9.0[a]	19.0	5.0	Feb., 1952
	3.0 × 2.8 × 1.8	Deficient	Deficient	63	8.0[a]	18.0	5.0	Feb., 1952
	4.0 × 3.5 × 2.0	Deficient	Deficient	74	8.0[a]	17.0	4.5	Feb., 1952
	4.0 × 3.5 × 2.0	Deficient	Deficient	75	8.0[a]	15.0	3.5	Feb., 1952
	3.0 × 2.6 × 1.6	Deficient	Deficient	87	11.0	21.0	5.0	Apr., 1952
	3.0 × 2.6 × 1.6	Deficient	Deficient	89	10.0	17.5	3.8	Apr., 1952
	2.0 × 1.2 × 1.6	Deficient	Deficient	95	8.0	13.5	2.8	May, 1952
	2.4 × 2.0 × 0.1	Deficient	Deficient	100	8.5	15.0	3.3	May, 1952
	2.4 × 2.0 × 0.1	Deficient	Deficient	105	8.0	18.5	5.3	May, 1952
Transplanted mammary tumor	4.0 × 1.5 × 1.5	Deficient	Deficient	38	7.5	15.0	3.8	Feb., 1952
	4.0 × 1.5 × 1.5	Deficient	Deficient	39	8.0	16.0	4.0	Feb., 1952
	4.0 × 1.5 × 1.5	Deficient	Deficient	40	10.0	18.5	4.2	Feb., 1952
	4.0 × 1.5 × 1.5	Deficient	Deficient	41	8.5	15.5	3.5	Feb., 1952
	4.0 × 1.5 × 1.5	Deficient	Deficient	42	9.5	9.0	0	Feb., 1952
	2.0 × 1.0 × 0.5	Deficient	Deficient	43	9.0	15.5	3.3	Feb., 1952
	2.0 × 1.0 × 0.5	Deficient	Deficient	44	9.0	14.0	2.5	Feb., 1952
	2.0 × 1.0 × 0.5	Deficient	Deficient	45	10.5	17.0	3.3	Feb., 1952
	2.0 × 1.0 × 0.5	Deficient	Deficient	46	11.5	19.5	4.0	Feb., 1952
	2.0 × 1.0 × 0.5	Deficient	Deficient	47	11.0	17.5	3.3	Feb., 1952
	2.0 × 1.0 × 0.5	Deficient	Deficient	48	10.5	17.0	3.3	Feb., 1952
	2.0 × 1.0 × 0.5	Deficient	Deficient	49	10.5	16.0	2.7	Feb., 1952

[a] Young started when 23 days old.

group of young was obtained from females treated exactly as described except that no transplantation was carried out. The average rate of growth of young from the tumor-bearing mothers was 3.2 g., and that for those from the normal mothers was 3.3 g. (compare table 5).

Trials With Urethane-Induced Lung Adenomas.—A large number (one hundred) of Swiss females 6 weeks of age were given urethane, 2 g. per kilo, in a single intraperitoneal dose. Four months later, autopsy of 12 of these animals showed that the nodules in the lungs were present in all (average number 37, range 7–54), and that they were quite large as such lesions go. Six of the surviving animals were then fed the deficient ration, and a week later, males were introduced to them. The experiment was then conducted as described for mice with spontaneous mammary cancers. Two experiments were performed. In the first, the average gain per week of the young was 3.8 g., and in the second it was 4.0 g. The presence of relatively large nodules in the lungs of the mothers was established by autopsy shortly after the litters were weaned.

Although these data indicated that no significant synthesis of vitamin B_{12} could be attributed to these adenomas, there was still some doubt about it. The females bearing the urethane-induced tumors did not prove to be very fecund, and frequently ate their young during the suckling period. In several litters the individuals were all noticeably undersized at 21 days of age. In order to obtain weanlings large enough to survive when placed on the deficient ration in individual cages, the time of weaning had to be prolonged to 23 or 24 days (cf. Ref. 14). These considerations, taken along with the fact that the average rate of gain was slightly above the values of table 1 in both experiments, may indicate that some synthesis of vitamin B_{12} was conducted by the adenomas.

Discussion

If the present evidence is accepted as showing that spontaneous mammary tumors synthesize large amounts of vitamin B_{12}, it would constitute the first demonstration of a qualitative metabolic difference between normal and neoplastic tissue, and would likewise point directly to a rational pathway of chemotherapeutic attack. Let us therefore attempt to see whether some other explanation can be attached to the observed facts. The data presented seem adequate to show that mice born and raised on a diet deficient in vitamin B_{12} grew faster if their mothers carried spontaneous mammary tumors. Furthermore, such mice grew almost as fast as similar individuals, the mothers of which had received this vitamin in their food, and two-thirds as fast as mice given continuously a superabundant supply of B_{12}.

It may be that the apparent synthesis of the vitamin was due to growth of B_{12}-forming micro-organisms in necrotic portions of the cancers. This

possibility was constantly held in mind, and because of it seemingly uninfected growths only were used. It may also be that necrosis of the older portions of the tumor was occurring with the liberation of vitamin B_{12} into the circulation of the animals. This possibility cannot be excluded. Nevertheless, the failure of transplanted mammary tumors to give evidence of vitamin synthesis would seem to be a control of this unknown because the necrosis in the transplant should have been at least as much as that in the spontaneous cancers. The same argument might also apply against the idea that the vitamin arose from microbial action.

It may be that normal mammary tissue carried out the synthesis of vitamin B_{12}, but, because it represented only a small portion of the total weight of the animal, the amount formed was not sufficient to meet all needs. When the cancer arose the proportion of this kind of tissue would be greatly increased, and might thus suffice to meet the demand for B_{12}. The B_{12}-manufacturing facilities of the mouse would thus merely be increased incidentally with the propagation of cells derived from mammary tissue. This also is a possibility which cannot be excluded, because it is not possible to grow mammary tissues in a chemically defined medium, and to measure any synthesis of the vitamin. There is no evidence to show that a normal mouse cannot form small amounts of vitamin B_{12}. All that preceding nutritional investigations have revealed is that such an animal cannot make enough of it to sustain normal function, and, in some cases, life itself. However, in consideration of this possibility for explanation of the facts observed in the present work, the failure of the transplanted mammary cancers to show evidence of B_{12} synthesis would seem to argue in favor of the alternative hypothesis which is the thesis of this paper. Nevertheless, because of the limited trials with transplanted tumors in the present work, it would be unjustifiable to emphasize unduly the failure of such transplants to give evidence of vitamin B_{12} synthesis. More extensive experiments under a variety of conditions might conceivably uncover such evidence. In the present work this phase was not explored further because the first experiments yielded such a definitely negative answer.

The difference between spontaneous and transplanted mammary tumors which was, however, observed was indeed surprising and unexpected. One would think that there should be no difference. However, the observed facts may fortify a belief expressed by some investigators of the cancer problem that transplants are not necessarily equivalent to spontaneous growths.[16] Perhaps one may regard the change from normal to malignant cells as involving several mutations. One of these might be concerned with the acquisition of ability to synthesize vitamin B_{12}. However, it is not easy to understand why this capacity is not carried along in the transplanted cells.

The data seem to show that the spontaneous cancers probably do syn-

thesize vitamin B_{12} in relatively large amounts. The quantity is sufficient to meet the needs of the succeeding generation for growth. Furthermore, this is not a property of the tumors of one strain of mouse, because not only Swiss but C3H and C breeds behaved similarly. One wonders why the chemically induced lung adenomas did not give clear evidence for synthesis. If one sets aside the considerations mentioned in the experimental section, and concludes that there was indeed no synthesis discernible, then 2 possibilities remain; (*a*) this type of cancer does not resemble spontaneous mammary tumors in this respect; or (*b*) synthesis of B_{12} does take place, but because of the relatively small size (compared to the mammary tumors) of these lung nodules, the method used in the present investigation could not detect the amount formed. Clearly, enough of the vitamin must be formed to meet the needs of the entire forthcoming litter throughout the period of most rapid growth before the present technique would reveal it. The spontaneous mammary tumors probably succeed in this because of the large amount of tissue involved.

If it be concluded that the spontaneous cancers differ from normal tissue in possession of the ability to synthesize vitamin B_{12}, then the kinship of this phenomenon to some findings in the plant kingdom is striking enough to warrant consideration.[17] Thus, Gautheret[18] has found that the crown galls of plants differ from the corresponding normal tissue in being able to synthesize the growth hormone indole acetic acid. The normal tissue grew in a synthetic medium only when the hormone was added, whereas the cells from the galls grew well without it and, in fact, produced it. A second instance is that reported by Steward and Caplin[19] during the progress of the work described in the present paper. They observed that while carrot tissue could be cultivated *in vitro* in a synthetic medium, this was true only if the inoculum contained cambium cells. When care was taken to exclude cambium from the transplant, very little growth took place until an unknown substance present in coconut milk was added. Thus, the cambium could make its own supply of this coconut milk factor, whereas other cells of the carrot root could not.

In each of these 3 cases (mammary tumors vs. normal tissue, crown gall vs. normal tissue and cambium vs. neighboring cells) the undifferentiated tissue differed from the differentiated in having the ability to synthesize a biologically important substance needed by the differentiated structures, but not formed by them. In each case the actual biologically important compound was not the same, being vitamin B_{12} in the cancers, indole acetic acid in the galls and a new and unknown substance in the cambium. This may well indicate the diversity to be expected in future explorations in this region. Nevertheless, this correlation of undifferentiated structures and synthetic ability may well indicate the existence of some new and fundamental law.

SUMMARY

The growth rate of weanling mice fed a vitamin B_{12}-deficient ration was nearly as great as that of similar mice fed this diet plus the vitamin, provided that the mothers of these former weanlings carried spontaneous mammary cancers. This finding was interpreted to mean that the tumorous mothers synthesized vitamin B_{12} in sufficient quantity to meet the needs of their offspring. Control experiments were performed to show that such maternal storage, and the passing on to the offspring, probably did occur, as has also been indicated by previous work. Transplanted mammary tumors of the same strain failed to give evidence that they were synthesizing vitamin B_{12}. The phenomenon interpreted as synthesis of vitamin B_{12} was studied most in Swiss mice bearing spontaneous mammary tumors, but was also seen to a lesser extent in C3H and C strain mice similarly situated. The evidence for such synthesis by urethane-induced lung adenomas of Swiss mice was equivocal, possibly due to the small mass of such tumors. In the case of the spontaneous mammary tumors, some alternative explanations of the observed facts were discussed, but the idea of an acquired ability of the cancer to synthesize this vitamin seemed best to fit the observations. This difference in metabolic behavior between normal and neoplastic tissue, which thus seemed to be indicated for the first time, was recognized as a promising pathway to chemotherapeutic attempts, and it was discussed how previous demonstrations had allowed the formation of specific chemical compounds which, as antimetabolites to dimethyldiaminobenzene, harmed those living things which synthesize vitamin B_{12} and riboflavin, but left others untouched. The synthetical ability which the tumors were concluded to possess was recognized as akin to similar phenomena in the plant kingdom, namely (*a*) the synthetical ability of crown galls with respect to indole acetic acid, and (*b*) the similar capacity of cambium tissue to form an essential growth factor which neighboring cells require but do not make. In these 3 divergent systems it was seen that the undifferentiated tissue differed from the differentiated in possessing the ability to synthesize a particular biologically important compound.

* A short account of this study was presented at the Second International Biochemical Congress, Paris, July, 1952.

† With the technical assistance of G. Schaffner and P. N. Townsend.

[1] The terms "metabolic difference" and "metabolic reaction" as used in this paper signify those chemical reactions which proceed in living tissues by means of which various substances are synthesized or broken down. These terms are not meant to indicate only (as is sometimes assumed) those reactions associated with the burning of sugars or the destruction of amino acids.

[2] Woolley, D. W., *J. Biol. Chem.*, **183**, 495 (1950).

[3] Woolley, D. W., *Proc. Soc. Exptl. Biol. Med.*, **75**, 745 (1950).

[4] Woolley, D. W., *J. Exp. Med.*, **93**, 13 (1951).

[5] Woolley, D. W., *A Study of Antimetabolites*, John Wiley & Sons, New York, 1952.

[6] Woolley, D. W., and Pringle, A., *J. Biol. Chem.*, **194**, 729 (1952).

[7] It must be obvious that this is not the only consideration in chemotherapy. Thus, for example, the antimetabolite might be destroyed by some chemical reaction in the host. If this were to happen to any great extent, no chemotherapeutic effect would be possible.

[8] Lillie, R. J., Olsen, M. W., and Bird, H. R., *Proc. Soc. Exptl. Biol. Med.*, **72**, 598 (1949).

[9] Rubin, M., and Bird, H. R., *Poultry Sci.*, **26**, 309 (1947).

[10] The failure to recognize this tenacious storage of vitamin B_{12} was a cause of much confusion in the early work with vitamin B_{12} (1945–1948).

[11] Zucker, L. H., and Zucker, T. F., *Arch. Biochem.*, **16**, 115 (1948).

[12] Although one may ask whether the cages were changed often enough, the data of tables 1–4 will indicate that they were. If they had not been, it probably would not have been possible to demonstrate the difference in rate of growth between deficient and supplemented animals.

[13] Through the courtesy of Dr. H. B. Andervont of the National Cancer Institute, biopsy specimens from a number of these animals were examined histologically and were pronounced to be mammary adenocarcinomas.

[14] The ability of the mothers to nurse their young adequately was reflected in the condition of these young at weaning time (21 days). In the experiments to be described, care was taken to use those litters in which the young were all of approximately the same size as those from normal deficient mothers. Occasionally, litters were encountered in which the young were smaller than this at 21 days and in fact were still being nursed by their mothers. In these instances the tumors of the mothers were unusually large, e.g., $4 \times 3.5 \times 2.5$ cm. in one of the cases. In such instances, the weaning of the young was delayed for two or even three days. Experience with such litters showed that when placed on a vitamin B_{12}-deficient ration the rate of gain was usually less than that seen with robust litters. Nevertheless, the average rates of gain shown in table 4 included the values obtained from two of these litters which had been weaned late and had grown less rapidly.

[15] C3H mice bearing spontaneous mammary tumors were purchased from the Jackson Memorial Laboratories. The C strain animals were very generously supplied by Dr. E. S. Rogers of the Rockefeller Institute.

[16] The author is well aware that there is no experimental proof that transplants differ from spontaneous tumors. Nevertheless, he is also aware that many shreds of evidence have led a considerable number of experienced investigators to the view that there actually may be a difference. Perhaps the facts presented in this paper may be construed as tangible evidence in this direction.

[17] After the completion of this manuscript the author's attention was called to a paper by J. Lederberg (*Science*, **104**, 428 (1946)) in which the suggestion was made that a back mutant of a "leucineless" fungus, *Neurospora crassa*, was comparable to mammalian cancer, whereas the "leucineless" strain itself was comparable to normal mammalian tissue. If this view is valid, then the "malignant" mutant differed from the "normal" in possession of a leucine-synthesizing capacity.

[18] Gautheret, R. J., *Endeavour*, **9**, 21 (1950).

[19] Steward, F. C., and Caplin, S. M., Program, VII International Congress, International Society for Cell Biology; Yale University, September, 1950, p. 15.

PHENOMENA IN THE LIQUID-LIQUID CRITICAL REGION*

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In a "Communication to the Editor" of the *Journal of Colloid Science*,[1] the senior author discussed briefly the several phenomena associated with the liquid-liquid critical region, and remarked, among other things, that the rapid increase in molecular disorder occurring as a two-phase mixture of the critical composition is heated through the critical region implies a considerable increase in the entropy of the system. This increase would be revealed by the change in heat capacity of the system. We here present an exploratory investigation of this effect, to be followed soon by one of greater precision.

TABLE 1

HEAT CAPACITY IN CALS. PER MOLE, OF MIXTURES HAVING THE CRITICAL COMPOSITION

H_2O AND $(C_2H_5)_3N$			C_7F_{16} AND i-C_8H_{18}		
$t°$, AV.	$\Delta t°$	Cp	$t°$, AV.	$\Delta t°$	Cp
14.28	1.76	26.0	12.29	1.03	100.5
15.89	1.20	27.1	13.64	1.36	100.5
17.03	0.87	28.5	16.09	1.08	101
17.83	0.61	31.4	17.39	1.34	102.5
18.37	0.38	46.6	19.12	2.11	106
18.45	0.54	52.5	21.21	2.08	111
18.85	0.24	49.4	22.45	0.234	118
19.20	0.32	43.4	22.84	0.417	120
19.56	0.30	39.9	23.25	0.314	124.5
19.97	0.42	38.7	23.62	0.337	127
20.20	1.68	37.2	23.98	0.333	111
22.22	2.51	33.3	24.56	0.658	109
24.59	2.48	29.8	25.39	0.670	106.5
25.97	2.25	27.5	25.97	0.645	105.5
27.74	1.59	26.8	26.45	0.431	105
29.88	2.89	25.4	27.35	0.551	104.5
32.26	2.12	24.2	27.83	0.555	104.5
			28.54	1.10	105
			29.46	1.09	106

First we wish to comment upon the thermodynamics of the process in somewhat more detail than in the communication mentioned above. To avoid the complications caused by polar, and particularly hydrogen-bonded, molecules we choose non-polar components. In order to have limited miscibility, the intermolecular potentials within the two pure liquids must be very different. The potential between the unlike molecules in the solution is approximately the geometric mean of the like po-

tentials, which is less than the arithmetic mean. Therefore, as mutual solubility increases with increasing thermal motion, 1–1 and 2–2 neighbors are replaced by 1–2 neighbors, with a decrease in the total intermolecular potential energy of each phase, causing expansion and absorption of heat. The connection between these two quantities is well recognized in the theory of regular solutions.[2] Both increase more and more rapidly as the top of the liquid-liquid solubility curve is approached, but still continue, although at a greatly reduced rate, after the disappearance of the meniscus, because the mixing is still far from complete, as shown by the turbidity that persists in gradually decreasing amount as the temperature is further raised.

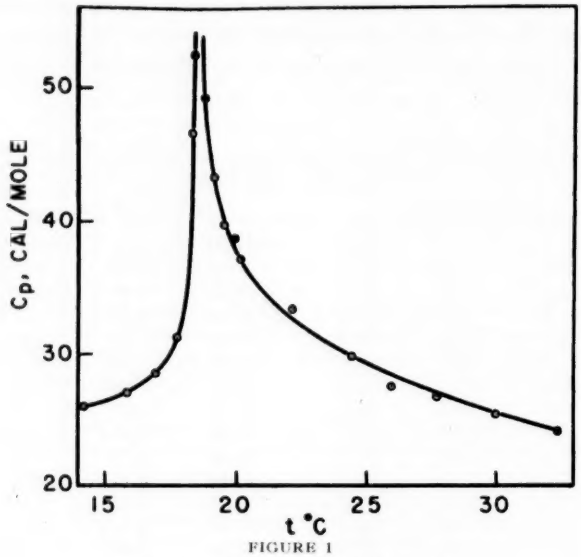

FIGURE 1

Critical mixture of H_2O and $(C_2H_5)_3N$.

Conversely, on lowering the temperature of a mixture of the critical composition well above the critical region, there occur, first, aggregations of like molecules of continually increasing size, as revealed in the beautiful work of Zimm.[3] These remain suspended by Brownian movement until they become sufficiently large for the gravitational field to separate them into an upper and a lower phase. It seems obvious that the precise temperature at which this occurs is a function of the density difference and the strength of the field, and might be measurably raised in a centrifugal field. The role of gravitation in the critical region of a one-component system has recently been strikingly shown by Murray and Mason.[4] Furthermore, there must

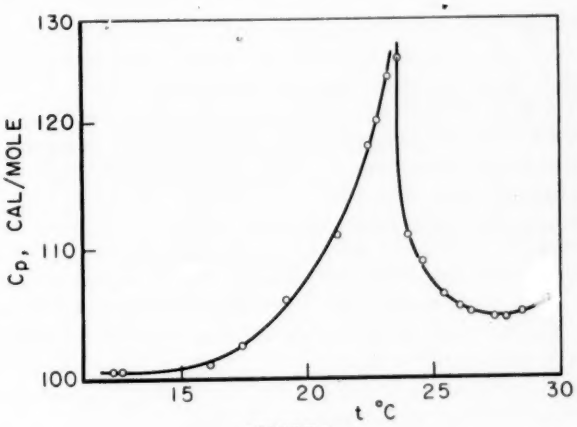

FIGURE 2
Critical mixture of C_7F_{16} and i-C_8H_{18}.

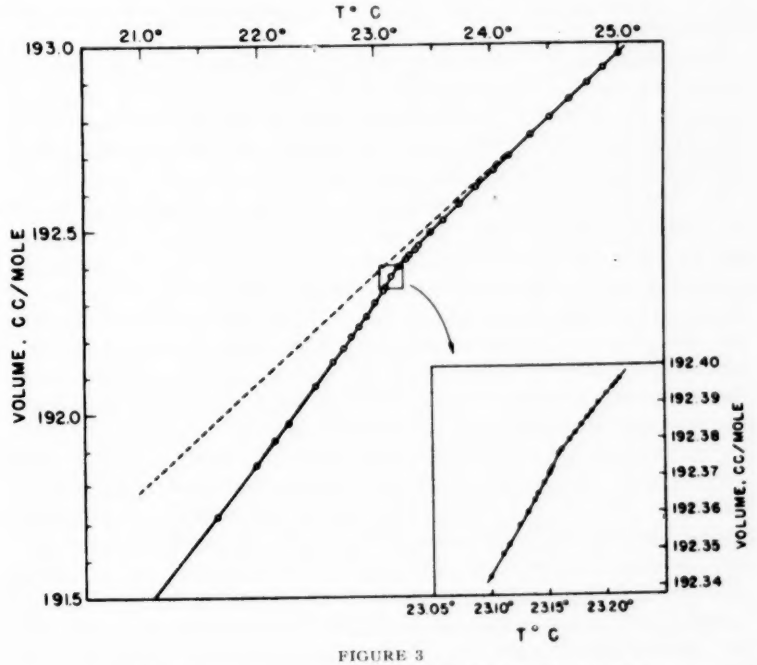

FIGURE 3
Molal volumes mixture of C_7F_{16} with i-C_8H_{18} in critical region.

be a sort of Brownian movement perpendicular to the plane of the interface just below the temperature at which it appears. The microscopic area of the interface must be greater than the macroscopic.

We have demonstrated the changes in heat capacity and volume in accordance with the above picture, using perfluoronormal heptane and 2,2,4-trimethylpentane, "iso-octane." Their mutual solubility curve had been determined in this laboratory.[5]

The calorimeter vessel had a capacity of 500 cc. Temperatures were measured with a calibrated resistance thermometer and a Miller bridge. The energy input was electrical, measured by the usual potentiometric method. The vessel was nearly filled with a mixture of the critical composition, and electrical energy added sufficient to cause the successive increments in temperature headed "Δt" in table 1. The listed values, "t, av.," are the means of the initial and final temperatures for each increment. The stirring was adequate to insure equilibrium.

In order to test the procedure, and to gain an idea of the magnitude of the effect, we first used the system water–triethylamine, for which the solubility curve has been accurately determined by Kohler.[6] Table 1 gives the heat capacities per mole of mixture for both systems, and they are plotted in figures 1 and 2.

The rising branch of each curve was of the expected form, but it is the falling branch that is particularly interesting. The descent continues smoothly through a number of degrees, with no discontinuity such as implied in the picture of a "derby hat." It is quite consistent with the gradual decrease in light scattering in the same region.

The volume changes were observed in a dilatometer with a "bulb" 33 cm. long and 5 mm. in internal diameter, bent into a circular arc and containing a steel ball for magnetic stirring. The capillary stem had an internal diameter of 0.56 mm., and was sealed on perpendicular to the plane of the arc of the bulb. A mixture of the critical composition was warmed to give one phase and introduced into the dilatometer through a fine steel capillary, and this was placed in an insulated bath of 33 gallons of water. Its temperature remained constant within 0.002° for periods far in excess of those required for equilibrium. The mixture was thoroughly stirred at the top temperature, the level was adjusted high in the capillary, and readings were taken at a series of stationary temperatures produced by additions of ice water. After several practice runs, the one plotted in figure 3 was made, lasting 8 hours. The results close to the critical are represented on a tenfold larger scale in the inset. The dotted tangent is drawn to make more evident the divergence between the portions above and below the critical "point," and especially the convexity above, corresponding to the continuance of the mixing process. After this is complete, it is to be expected that the curve would show the usual slight upward concavity. It is ob-

vious that the slope of this curve plotted against temperature would be, like figures 1 and 2, of the "lambda" type.

We will await the results of the precision study now under way before attempting to reduce the qualitative picture we have given of the balance between energy and entropy to quantitative terms.

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CHROMATOGRAPHIC SEPARATION AND IDENTIFICATION OF SOME PEPTIDES IN PARTIAL HYDROLYSATES OF GELATIN

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Recently we have been engaged in a study of the chemical structure of collagen and gelatin with the object of determining the sequence of the amino acid residues in the polypeptide chains of these proteins. In the course of this study we have made considerable progress in the chromatographic analysis of complex mixtures of peptides and we have isolated and identified several simple peptides which occur in partial hydrolysates of gelatin. The initial separation of the mixture into zones of one or more peptides has been made on a column of ion exchange resin; further separation of the peptides in each zone has been achieved by chromatographing in the form of dinitrophenyl (DNP) peptides on columns of silicic acid-Celite. It is to be hoped that the particular combination of chromatographic methods which has been successfully used in the present study will be helpful in the resolution of the complex mixtures which result from the partial hydrolysis of other proteins.

Although the amino acid composition of collagen and of its derivative, gelatin, is fairly well established by good analyses[1] (which have shown that there is virtually no difference in the amino acid composition of the two), relatively little is known about the sequences of amino acid residues in these proteins. In 1936 Grassman and Riederle[2] succeeded in isolating the tripeptide lysylprolylglycine (lys-pro-gly)[3] from partial hydrolysates of

gelatin. Later Gordon, Martin, and Synge[4] obtained evidence for sequences which contained glycine-leucine, proline-alanine, proline-glycine, and proline-alanine-glycine but the exact sequences were not determined. Very recently, Heyns, Anders, and Becker[5] found the peptides glu-gly, ala-gly, gly-asp, ala-ala-gly, and ala-(gly, glu) in partial hydrolysates of gelatin.

The sequences of the amino acid residues in collagen and gelatin are especially pertinent to the evaluation of the structures which have been put forward on the basis of data from amino acid composition and x-ray diffraction patterns. Some years ago Astbury[6] suggested that in collagen the sequence will be —P—G—R—P—G—R—P—G—R— where P, with the exception of one residue in 18, stands for either proline or hydroxyproline, G for glycine, and R for one or other of the remaining residues. Recently, Pauling and Corey[7] have described a definite configuration for the polypeptide chains in collagen and gelatin which embodies the same sequence of residues. In both of these quite different structures the packing of the residues appears to require that proline (or hydroxyproline) and glycine be present in the sequence of a prolyl (or hydroxyprolyl) glycine peptide and not in the reverse order. Thus far, only lys-pro-gly, isolated by Grassman and Riederle, and gly-asp, isolated by Heyns, Anders, and Becker, are in definite accord with this requirement.

General Experimental Procedure

The peptides which were present in the partial hydrolysates were resolved into a number of discrete zones by means of the ion exchanger Dowex-50. The procedure for this separation differed from that of Moore and Stein[8] for the analysis of amino acids on 100-cm. columns only in that a temperature of 37.5° was maintained throughout the chromatogram. The effectiveness of the separation was assessed by applying the ninhydrin procedure of Moore and Stein[9] to every other one of the approximately 1-ml. fractions of effluent. We have tried to improve the separation of the peptide zones by altering the conditions of development but none of our modifications seemed to offer any obvious advantage over the procedure which is indicated above.

Because the partial hydrolysate of a protein is so complex a mixture, one would not expect the relatively few peptide zones which can be isolated from the ion exchange column to be composed individually of a single compound. One is, therefore, faced with the choice of a method for the separation, identification, and estimation of the components of the mixtures. This choice is restricted because of the salt content of the buffers which are required by the ion exchange method. The quantity of salt is manyfold greater than the quantity of peptide, and hence the salt would interfere with attempts at further chromatographic separation, say by paper chromatography. We have resolved this difficulty by converting the materials

in the zones to the DNP-derivatives. The conversion to the DNP-peptides is not affected by the presence of the salts and the separation of salts and DNP-peptides occurs when the latter are extracted from the acidified reaction mixture with ether or ethyl acetate. The resolution of the DNP-peptides was then carried out on columns of silicic acid-Celite. The methodology was essentially that of Green and Kay[10] for the separation of DNP-amino acids except that stronger developers were required. Very satisfactory separations have been obtained by starting the development of the chromatogram with a solution which contains 8 volume per cent of acetic acid and 4 volume per cent of acetone in ligroin (abbreviated 8AA-4A-L)[10] and then changing to 3AA-15A-L, 4AA-20A-L, or 5AA-25A-L as may be necessary to develop strongly adsorbed DNP-peptides. If the zones isolated in this manner proved to be heterogeneous, 2AA-10A-B or 3AA-15A-B (B = benzene) were valuable in achieving further separations.

The constitution of the DNP-peptides so separated has been established by hydrolyzing them completely and determining the N-terminal amino acid by the method of Green and Kay.[10] The remaining amino acids of the peptide are present in the hydrolysate as the free amino acids; they were identified by dinitrophenylating them and identifying the DNP-amino acids so produced. The quantities were determined spectrophotometrically in glacial acetic acid.

RESULTS

Initial Separation of Peptides on Ion Exchange Chromatograms.—Figure 1 compares the results of identical chromatograms of a complete and of a partial hydrolysate of about 12 mg. of gelatin. In this figure, the optical densities of the various fractions after application of the ninhydrin procedure are plotted against the fraction number.

The positions of those zones which emerge during the development with pH-3.42 buffer are in good agreement with the positions of the amino acids in figure 1 of Moore and Stein's paper,[8] and very likely in this region the zones from the partial hydrolysate contain mainly free amino acids. That peptides may be present, however, is shown by the fact that the ninhydrin color from the proline zone of the partial hydrolysate definitely is not the usual brown-red color given by proline itself; the abnormal color indicates the presence of material with a free amino group. The difference in the chromatograms throughout the development with the other buffers clearly proves the presence of peptides in the zones of the partial hydrolysate.

The chromatograms which have been described were intended largely to explore the potentialities of the ion exchanger as a means of initial separation of the peptides in the partial hydrolysate: the results were encouraging. However, further investigation was necessary in order to determine whether or not other conditions of hydrolysis might yield more peptide zones or greater amounts of those already observed.

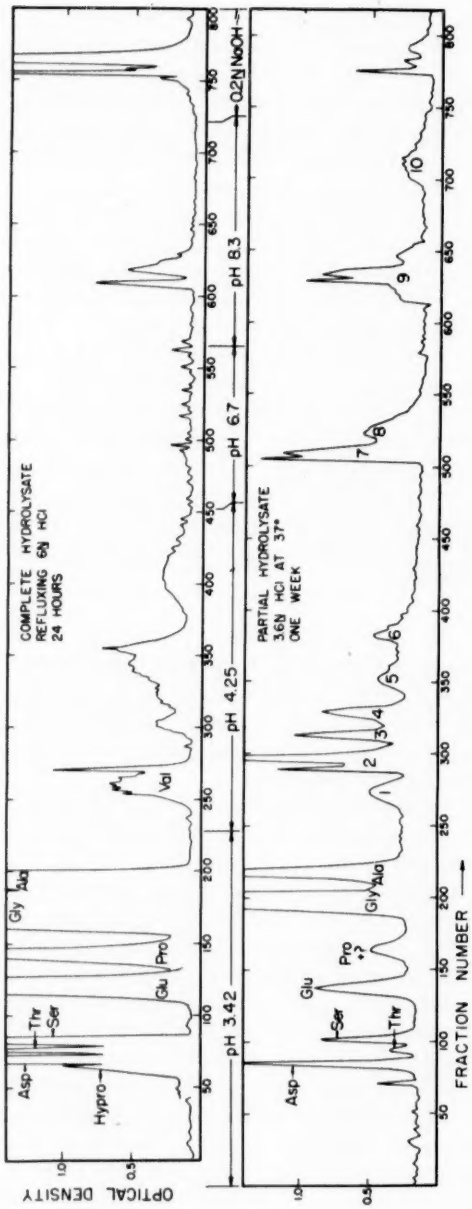

FIGURE 1

Comparison of chromatograms of partial and complete hydrolysates of gelatin on Dowex-50.

It was found that longer hydrolyses in 3.6 *N* hydrochloric acid at 37° exhibited no obvious advantages over the one-week hydrolysis as far as a source of peptides was concerned. A basic hydrolysate prepared essentially in the manner of Heyns, Anders, and Becker[5, 11] yielded a chromatogram which was very different from that of figure 1. During hydrolysis in dilute oxalic acid the preferential release of aspartic acid is a prominent feature, as reported by Partridge and Davis.[12]

Further Separation and Identification of Peptides.—In order to obtain sufficient material for a definitive study of the peptides in the various zones, the partial hydrolysate of about 250 mg. of gelatin which had been hydrolyzed in 3.6 *N* hydrochloric acid at 37° for one week was chromatographed on a large ion exchange column. The investigation of those zones of this large-scale chromatogram which are equivalent to zones 1, 2 and 3 in figure 1 is essentially complete.

Zone 1.—This zone consists almost entirely of thr-gly. The quantity is appreciable and as a minimum accounts for 40% of the threonine in gelatin on the basis of the analysis given by Tristram.[1] If corrections are applied, 55% of the threonine may be accounted for in this form. This correction which may or may not be applicable is largely a correction for incomplete dinitrophenylation of the peptides from the ion exchanger. In a series of experiments with known peptides a yield of more than 75% was not obtained despite wide variations in the conditions of dinitrophenylation; the presence of buffer salts in the reaction mixture was without effect on the yield. Whether such a correction is applicable to all peptides is open to question.

Zone 1a.—Due to incidental differences in the way in which the large- and small-scale ion exchange chromatograms were run, the large chromatogram exhibited a minor zone between zones 1 and 2. When the DNP-peptides which resulted from the dinitrophenylation of this minor zone were chromatographed, five zones separated, and two of these were present in sufficient amount to warrant further study.

The more strongly adsorbed zone contained a glycyl peptide or peptides of which the other constituent amino acids were glycine, alanine, proline, and hydroxyproline. Quantitatively, the N-terminal glycine, the other glycine, and the proline were present in equimolar proportions and in twice the molar amount of both the alanine and hydroxyproline. One might interpret these results to mean that a mixture of gly-(gly, pro, ala) and gly-(gly, pro, hypro) is present. However, on the basis of the chromatographic properties of known DNP-peptides such a conclusion is untenable: a more reasonable inference is that the mixture contains gly-(gly, pro, ala, gly) and gly-(pro, hypro).

The less strongly adsorbed zone was shown to be glu-gly. Some glu-gly is also present in zone 2.

Zone 2.—The initial separation of the DNP-peptides from zone 2 yielded five definite bands on the chromatogram. For simplicity they will be designated as zones 2a, 2b, etc., in the order of decreasing adsorption affinity.

Zone 2a is present in relatively minor amount and appears to contain gly-(hypro, gly) as the main constituent. No definite conclusions can be made about the other peptides which are present in still smaller amounts.

Zone 2b is the main peptide in zone 2 and contains hypro-gly in amount equal to 13 to 19% of the hydroxyproline in gelatin. Although hydrolysis alters or destroys DNP-proline and DNP-hydroxyproline more easily than other DNP-amino acids, the detection of DNP-prolyl or DNP-hydroxyprolyl peptides is very simple because the spectra of these *imino* peptides differ from those of the *amino* peptides. Whereas the spectra of the DNP-amino acids in glacial acetic acid have the main maximum at 338 to 342 mμ, the spectrum of DNP-proline has a maximum at 360 mμ and that of DNP-hydroxyproline at 355 mμ and the latter two, in addition, have no inflection in the region from 380 to 420 mμ.[13] The spectra of DNP-prolyl and DNP-hydroxyprolyl peptides show the characteristics of DNP-proline and DNP-hydroxyproline.

Zone 2c was composed of a mixture of three peptides. Rechromatographing with 3AA-15A-B separated ser-gly from a mixture of gly-glu and glu-gly. The serine in ser-gly accounts for 23 to 33% of the serine in gelatin.

Zone 2d contains glu-ala and a small amount of ala-gly.

Zone 2e may contain thr-ala but the amount is small.

Zone 3.—Six definite zones separated when the DNP-peptides were chromatographed. So far only the main peptide, ala-gly, has been completely investigated.

Discussion

The work of Grassman and Riederle,[2] of Heyns, Anders, and Becker[5] and of the present study has resulted in the identification of an appreciable number of amino acid sequences in gelatin: Grassman and Riederle isolated lys-pro-gly; Heyns, Anders, and Becker found glu-gly, ala-gly, gly-asp, ala-ala-gly, and ala-(gly, glu); and we have definitely identified thr-gly, glu-gly, glu-ala, gly-glu, ser-gly, hypro-gly, and ala-gly and possibly have also isolated thr-ala, gly-(pro, hypro), gly-(gly, gly, ala, pro) and gly-(gly, hypro). If we compare these peptides with the sequence —R—pro-(or hypro)-gly- which is suggested by the structures of Astbury[6] and of Pauling and Corey,[7] it will be observed that the lys-pro-gly, gly-asp, gly-glu, and hypro-gly fit the sequence whereas glu-gly, ala-gly, ala-ala-gly, ala-(gly, glu), thr-gly, glu-ala, and ser-gly do not. At first glance these data appear to cast doubt on the proposed structures. However, the most reliable analyses of gelatin[1] show that although one-third of the residues are

indeed glycine, as required by the structures, only about one-fourth instead of one-third are proline and hydroxyproline. Hence, at a maximum only three-fourths of the protein could have the sequence—R—pro (or hypro)-gly- and the remaining one-fourth would have no predictable sequence. Accordingly, we would expect to find appreciable amounts of peptides whose sequences do not agree with the over-all structures which have been proposed. The available data are still too meager to permit a definite decision as to the essential correctness or incorrectness of the suggested structures. It will be necessary to accumulate much more information, and we feel especially that quantitative information is essential. We believe that the isolation of trace amounts of various peptides from the partial hydrolysate of a protein is of dubious significance in elucidating the structure of a protein, but that a major step in the determination of structure will have been taken when the greater part of its content of amino acids is accounted for in the form of various peptides in a partial hydrolysate. To this end, our experiments have been designed to yield at least roughly quantitative data, and we have indicated the quantities which have been isolated in those instances in which the amount was appreciable.

It is probable that the effectiveness of the combination of chromatographic methods which we have used rests in the differences in the mechanisms by which separations are effected by the ion exchanger and by the silicic acid. During the chromatography of the free peptides the anchoring groups, both ionic and non-ionic, which operate in the adsorption process on the ion exchanger, are different from those anchoring groups which are effective in the adsorption of the DNP-peptides on silicic acid. Thus, although a group of peptides may not be separable on the ion exchanger under the conditions which have been used, their conversion to the DNP-derivatives so alters their properties that they behave very dissimilarly when chromatographed on silicic acid.

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* Contribution No. 1748.

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VIABILITY OF HYBRIDS BETWEEN LOCAL POPULATIONS OF DROSOPHILA PSEUDOOBSCURA

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Artificial populations of *Drosophila pseudoobscura* have been made in laboratory experiments which involved two or more chromosomal types differing in the gene arrangements in the third chromosomes. Provided that the chromosomal types are derived from the population of the same geographic locality, the artificial populations eventually reach equilibria at which each type continues to occur with a certain characteristic frequency.[1, 2] The attainment of such equilibria means that the structural heterozygotes which carry two chromosomes with different gene arrangement are heterotic, i.e., possess higher adaptive values than do the corresponding homozygotes. If, however, the chromosomal types are derived from geographically remote populations, then some of the chromosomes may eventually be eliminated and others established.[3, 4] This has been interpreted to mean that the heterosis is produced by juxtaposition of chromosomes which carry different gene complexes coadapted, fitted together, by natural selection in the process of evolution. Chromosomes found in geographically remote populations need not be coadapted, and the structural heterozygotes obtained in interracial crosses need not show heterosis.

In the above experiments, the cytologically visible characteristics of the chromosomes were used as markers which permitted an observer to follow the fates of different gene complexes in populations. It seems, however, probable that heterosis is the normal state of natural populations of most sexually reproducing diploid organisms, regardless of whether their chromosomes are or are not visibly polymorphic. The experiments reported in the present article were designed to test this hypothesis.

Material and Technique.—The material used for the experiments described below consisted of four strains of *Drosophila pseudoobscura* from each of the following localities: Piñon Flats, Mount San Jacinto, California; Mather, Sierra Nevada, California; Bryce National Park, Utah; Ferron, Utah, and Black Canyon of the Gunnison National Monument,

Colorado. All 20 strains were cytologically uniform, being homozygous for the Arrowhead gene arrangement in the third chromosomes. As a control, a strain homozygous for the third chromosome recessive mutant orange eye was used.

Three kinds of crosses were made, namely: (1) between strains derived from the same locality, (2) between strains derived from different localities, and (3) between the F_1 hybrids. All crosses were made using mixtures of females from some and males from other strains from the localities described, so that the eggs obtained represented populations of known geographic origin. The parent flies were placed for 24 hours in glass vials containing paper spoons with the regular cream of wheat-molasses medium. The spoons with eggs deposited on them were inspected under a binocular microscope, and the freshly hatched larvae were transferred with the aid of a needle into vials containing 4 cc. of synthetic food medium described by Kalmus,[5] on the surface of which had been placed 0.1 cc. of freshly prepared 14% suspension of dry Fleischmann's yeast. In every vial there were placed 10 young larvae of the wild-type strain to be studied, and also 10 young larvae of the control (orange) strain. The vials were left at 25°C. until the adult flies hatched. The numbers of orange-eyed and of normal-eyed individuals among flies were determined and recorded.

The amount of food placed in the vials was made deliberately insufficient for the development of 20 larvae. The mean number of adult flies hatching per vial was 8.8. Summaries of the data obtained are presented in tables 1 to 4. The tables show the numbers of larvae of the different classes used and the percentages of these larvae developing into adult flies.

Control Experiments.—Larvae of the orange mutant are weaker than wild-type larvae. On the average, only 2.9 out of the 10 mutant larvae placed in each vial gave adults. Since the proportion of the control larvae surviving to become imagos is an index of the environmental rigors to which they are exposed in the overpopulated cultures, it is very important to know whether the survival in different experiments was uniform. The figures in the columns on the right in tables 1 to 4 were subjected to a statistical analysis. The first series of experiments, carried out between January and March of 1952, gave from 23.7 to 32.5% of survival of the orange class (tables 1 and 3). The second series, carried out in July and August of the same year, gave from 24.0 to 35.5% survival (tables 2 and 4). The chi-square for heterogeneity in the first series is 17.66, which for 11 degrees of freedom has a probability close to 0.09; and in the second series 25.26, which for 15 degrees of freedom has a probability of about 0.05. Combining all the experiments together, one gets a chi-square of 40.16, which for 27 degrees of freedom has a probability likewise of 0.05. The value of 0.05 is at the upper boundary of the conventional range of significance. It may, however, be noted that the greatest contribution to this chi-square

is made by a single series of experiments which gave an unusually low survival value of 23.7% (table 3). It can be concluded that the control experiments show a very slight, though probably real, heterogeneity.

The Local Populations and the F_1 Hybrids Between Them.—Tables 1 and 2 show that the wild-type flies obtained by intercrossing strains of similar geographic origin are less viable than the F_1 hybrids between the same strains. The per cent survival of the intrapopulational hybrids is 58.4 in table 1 and 60.6 in table 2, while the interpopulational hybrids gave 69.4% and 71.1% survival, respectively. The chi-square for the first series of experiments is 27.41, and for the second series 22.22. For one degree of freedom such values are highly significant.

TABLE 1

VIABILITY IN LOCAL POPULATIONS AND THEIR F_1 HYBRIDS

	EXPERIMENTAL		CONTROL	
LOCALITIES	NUMBER	PER CENT SURVIVAL	NUMBER	PER CENT SURVIVAL
Mather	350	60.3	350	30.0
Bryce	360	60.0	360	27.9
Gunnison	340	54.7	340	25.9
TOTAL	1050	58.4 Av.	1050	26.6 Av.
Mather × Bryce	360	72.2	360	32.5
Gunnison × Mather	360	66.1	360	30.3
Gunnison × Bryce	360	69.7	360	26.4
TOTAL	1080	69.4 Av.	1080	29.8 Av

TABLE 2

VIABILITY IN LOCAL POPULATIONS AND THEIR F_1 HYBRIDS

	EXPERIMENTAL		CONTROL	
LOCALITIES	NUMBER	PER CENT SURVIVAL	NUMBER	PER CENT SURVIVAL
Piñon	220	54.1	220	31.3
Bryce	240	69.5	240	31.7
Ferron	230	62.1	230	34.7
Gunnison	240	56.2	240	26.6
TOTAL	930	60.6 Av.	930	31.1 Av.
Piñon × Bryce	220	79.8	220	28.1
Piñon × Ferron	230	77.8	230	25.6
Piñon × Gunnison	220	69.3	220	30.0
Ferron × Gunnison	240	57.7	240	32.5
TOTAL	910	71.1 Av.	910	28.0 Av.

The Local Populations and the F_2 Hybrids Between Them.—The F_1 hybrid flies obtained in the experiments just described were inbred, and F_2 progenies were exposed to competition with the intrapopulational hybrids and with the orange mutant. Tables 3 and 4 summarize the results. The intrapopulational hybrids showed the same survival ability as in the preceding experiments—55.4% and 62.1%. But the F_2 hybrids between the

populations proved now to be less viable, not only than their F_1 parents, but even than the local populations themselves. The survival values obtained for the F_2 hybrids are 47.5 and 50.4%. The differences between the intrapopulational and the F_2 interpopulational hybrids are statistically quite significant, the chi-squares being 19.79 and 21.86 for the two experiments, respectively. For one degree of freedom such values will occur by chance less than once in 1000 trials.

TABLE 3

VIABILITY OF LOCAL POPULATIONS AND THEIR F_2 HYBRIDS

	EXPERIMENTAL		CONTROL	
LOCALITIES	NUMBER	PER CENT SURVIVAL	NUMBER	PER CENT SURVIVAL
Mather	500	62.0	500	29.0
Bryce	520	54.0	520	23.7
Gunnison	520	50.4	520	30.0
TOTAL	1540	55.4 Av.	1540	27.5 Av.
Mather × Bryce	510	57.8	510	28.9
Gunnison × Mather	520	41.9	520	25.8
Gunnison × Bryce	540	43.0	540	29.8
TOTAL	1570	47.5 Av.	1570	28.0 Av.

TABLE 4

VIABILITY OF LOCAL POPULATIONS AND THEIR F_2 HYBRIDS

	EXPERIMENTAL		CONTROL	
LOCALITIES	NUMBER	PER CENT SURVIVAL	NUMBER	PER CENT SURVIVAL
Piñon	210	52.4	210	27.6
Bryce	180	66.1	180	35.5
Ferron	180	59.4	180	34.4
Gunnison	200	71.6	200	35.5
TOTAL	770	62.1 Av.	770	33.4 Av.
Piñon × Bryce	200	47.0	200	26.5
Piñon × Ferron	200	43.5	200	24.0
Piñon × Gunnison	200	52.5	200	30.0
Ferron × Gunnison	190	58.9	190	30.5
TOTAL	790	50.4 Av.	790	27.5 Av.

Conclusions.—It is evident that the gene recombination in *Drosophila pseudoobscura* leads to a significant loss of the viability among the hybrids, compared to the local populations themselves. This finding is in agreement with the results obtained by Dobzhansky and others[1-4] who studied the phenomena of heterosis in structural heterozygotes in the same species. The genotype of each local population or race evidently represents an integrated adaptive system, the different parts of which are mutually adjusted or coadapted by natural selection. Such systems are broken down by recombination in hybrids. This is, of course, essentially the same phenomenon which is observed in the progeny of many fertile interspecific hybrids.

The superiority of the F_1 hybrids over their parents is more difficult to account for. Granted that the genotype of each race is a coherent adaptive system, at least the F_1 hybrid females will have two full haploid sets of internally balanced chromosomes. This might be expected to make their F_1 hybrids equal in viability to the parents, but not superior to the latter. Of course, the F_1 hybrids are heterozygotes for more loci than are individuals within each local population, even though the latter come from intercrossing of different strains. Some early students of heterosis supposed that heterozygosis is *per se* a viability stimulus, and the data presented above agree with this view. Before it is adopted one must, however, exclude certain alternative explanations. It may be noted that three of the local populations used—namely, Bryce, Ferron, and Gunnison—consist of flies a majority of which are homozygous for the Arrowhead gene arrangement in the third chromosome, while in two populations, Piñon and Mather, homozygous Arrowhead flies are a minority.[6] Intercrossing strains with these gene arrangements from Piñon and from Mather may, then, give populations with a viability lower than that in the natural populations of these localities which contain other chromosome types as well. Indeed, tables 2 and 4 show that the viability of Piñon flies is lower than that of Bryce, Ferron, and Gunnison flies. However, the viability of the Mather flies (tables 1 and 3) is not only not lower but ostensibly even higher than that of Bryce and Gunnison, although the differences are not significant statistically.

It must also be emphasized that the experiments reported here deal with the viability of the different groups of flies between the stages of a freshly hatched larva and the ecclosion of the adult fly from the pupa. They tell us nothing about other properties, such as the longevity, fecundity, and sexual activity of the adult flies, which are important in determining the adaptive value of carriers of a given genotype. It is consequently premature to speak of heterosis among the F_1 hybrids, and of its absence among F_2 hybrids between the populations studied. Nevertheless, the observed variations in larval and pupal viability are very real, and further studies are needed to understand their causation.

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ON COMPLEXES WITH OPERATORS

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In the algebraic topology of covering spaces one is led to a study of complexes with groups or rings of operators corresponding to the covering transformations. Such complexes with operators have been investigated[1] in connection with the *relations between the fundamental group and the homology structure of an aspherical space*, and with the *cohomology theory of groups and rings*. For this purpose, various kinds of cohomology groups, with special properties relative to the operators, had to be considered. In this note a fundamental isomorphism for such cohomology groups is established (Theorem 1) and applications to different problems are sketched: cohomology of groups, generalization of the "transfer" (Verlagerung) of a group into a subgroup; cohomology groups of general covering spaces; "derivations" in abstract groups[2] and end-points of groups.

1. Complex.—1.1: By a complex $\mathfrak{C}$ we mean a sequence of Abelian groups C_p and of homomorphisms ∂ of C_{p+1} into C_p, $p = -1, 0, 1, 2, \ldots$, with $\partial\partial C_p = 0$ for $p > 0$ and $\partial C_0 = C_{-1}$. The complexes used in algebraic topology and in abstract algebra have, in most cases, the additional property that the groups C_p are free and have, for $p \geqslant 0$, a distinguished set of free generators corresponding to the cells or simplices (of dimension p) of a polyhedron, to the singular simplices of a space, to abstractly defined symbols, etc.[3]

1.2: Homology groups of $\mathfrak{C}$ are defined as usual by $H_p(\mathfrak{C}) = Z_p/\partial C_{p+1}$, $p \geqslant 0$, where Z_p is the kernel of ∂ in C_p. We shall deal mainly with *cohomology groups* of various types; we start with the "ordinary" cohomology groups of $\mathfrak{C}$ relative to an Abelian group J as coefficient group, denoted by $\check{H}^p(\mathfrak{C}, J)$ (the notation H^p will be used for certain "restricted" cohomology groups in complexes with operators): Let $\check{C}^p = \check{C}^p(\mathfrak{C}, J)$ be the group of all homomorphisms of C_p into J, and δ the homomorphism of $\check{C}^p$ into $\check{C}^{p+1}$ defined for $p \geqslant 0$ by $\delta f^p(c_{p+1}) = f^p(\partial c_{p+1})$ for all $f^p \in \check{C}^p$, $c_{p+1} \in C_{p+1}$, for $p = -1$ by $\delta\check{C}^{-1} = 0$. Then $\delta\delta\check{C}^p = 0$ for all p, and one defines $\check{H}^p(\mathfrak{C}, J) = \check{Z}^p/\delta\check{C}^{p-1}$, $p \geqslant 0$, $\check{Z}^p$ being the kernel of δ in $\check{C}^p$.

Elements $c_p \in C_p$ are called chains, $f^p \in \check{C}^p$ cochains, of dimension p.

2. Complex with Operators from a Ring R.—2.1: Let R be a ring with a unit e. A complex $\mathfrak{C}$ is called a *complex over R*, in short an *R-complex*, if all its groups C_p are R-modules[4] and all its homomorphisms ∂, R-homomorphisms. In this case, "*equivariant*" cohomology groups of $\mathfrak{C}$ relative to an R-module J are defined as follows: Let $C^p = C^p(\mathfrak{C}, J)$ be the group

of all R-homomorphisms of C_p into J (C^p is a subgroup of $\check{C}^p$ = group of all *additive* homomorphisms of C_p into J); it is easily seen that δ maps C^p into C^{p+1}, and we put $H^p(\mathfrak{C}, J) = Z^p/\delta C^{p-1}$, $p \geqslant 0$, where $Z^p = \check{Z}^p \cap C^p$ is the kernel of δ in C^p.

2.2: $\check{H}^p$ and H^p are examples of the following general kind of cohomology groups which are useful in several applications.

For a ring R and an (additive) Abelian group J, let $\Phi = \Phi(R, J)$ be the group of all additive homomorphisms of R into J, turned into an R-module by $(r' \cdot \varphi)(r) = \varphi(rr')$ for all $\varphi \in \Phi$, $r, r' \in R$. If $\mathfrak{C}$ is an R-complex, we define for any *submodule* Ψ of Φ cohomology groups $H_\Psi^p(\mathfrak{C}, J)$ "*of type* Ψ," as follows: Take all cochains $f^p \in \check{C}^p$ such that for each $c_p \in C_p$, $f^p(rc_p)$ considered as a function of $r \in R$ is an element of Ψ; they constitute a subgroup C_Ψ^p of C^p. Again δ maps C_Ψ^p into C_Ψ^{p+1}, and we put $H_\Psi^p(\mathfrak{C}, J) = Z_\Psi^p/\delta C_\Psi^{p-1}$, $p \geqslant 0$, where Z_Ψ^p is the kernel of δ in C_Ψ^p.

2.3: Examples of modules $\Psi \subset \Phi(R, J)$.

(*a*) If we take for Ψ the whole group Φ, there is no restriction on the cochains $f^p \in C_\Phi^p$; hence $H_\Phi^p(\mathfrak{C}, J) = \check{H}^p(\mathfrak{C}, J)$.

(*b*) Let J be an R-module, and Ψ_0 the group of all R-homomorphisms of R into J; $f^p \in C_{\Psi_0}^p$ is "equivariant," hence $H_{\Psi_0}^p(\mathfrak{C}, J) = H^p(\mathfrak{C}, J)$. Obviously $\Psi_0 \cong J$; an isomorphism q of J onto Ψ_0 is given by $q\tau(r) = r \cdot \tau$ for all $\tau \in J$, $r \in R$.

(*c*) Let Q be a *subring* of R, J a Q-module; we consider the group $\Psi = Q\text{-Hom}(R, J)$ of all Q-homomorphisms of R into J. In this case, a cochain $f^p \in C_\Psi^p$ is simply a Q-homomorphism of C_p into J. In other words, the groups $H_\Psi^p(\mathfrak{C}, J)$ are the equivariant cohomology groups of $\mathfrak{C}$ *considered as a complex over* Q. If $\mathfrak{C}$ as a Q-complex is denoted by $\mathfrak{C}_Q$, we thus have $H_\Psi^p(\mathfrak{C}, J) = H^p(\mathfrak{C}_Q, J)$.

(*d*) By R_A we denote the *group ring* of a group A, with integer coefficients. The elements $\varphi \in \Phi(R_A, J)$ can be considered as J-valued functions $\varphi(a)$ of $a \in A$. An R_A-module is an additive Abelian group in which A acts as a group of left operators.

The R_A-modules $\Psi \subset \Phi$ are groups of functions from A to J. E.g., let J be an R_A-module and define Ψ_0 as in (*b*): the elements of Ψ_0 are functions ψ from A to J such that $\psi(a) = a \cdot \psi(e)$. If A operates trivially in J ($a \cdot \tau = \tau$ for all $a \in A$, $\tau \in J$), then ψ is constant.

We shall use two other examples of function groups $\Psi \subset \Phi(R_A, J)$:

(*d'*) Let B be a subgroup of A, J an R_B-module. If in example (*c*) we take $R = R_A$, $Q = R_B$, we obtain the group ψ of functions ψ from A to J such that $\psi(ba) = b \cdot \psi(a)$ for all $b \in B$, $a \in A$. We call Ψ the R_A-*module induced by the* R_B-*module* J: the representation of A by operators of Ψ is exactly what one calls in the theory of linear representations the "*induced representation*," induced by the representation of B in J. If B operates trivially in J, the functions $\psi \in \Psi$ are constant on the right

cosets of A modulo B; the induced representation of A is then simply given by the translations of the right cosets.

(d'') For an arbitrary group A and an Abelian group J, let Ψ consist of those functions ψ for which $\psi(a) \neq 0$ *for at most a finite number of* $a \in A$. In an R_A-*complex* $\mathfrak{C}$, the corresponding groups $H_\Psi^p(\mathfrak{C}, J)$ are called the A-*finite cohomology groups* and are denoted by $H_{A\text{-fin}}^p(\mathfrak{C}, J)$; they are based upon cochains f^p such that, for any $c_p \in C_p$, $f^p(ac_p) \neq 0$ for a finite number of $a \in A$ only. *Remark:* If J is the additive group of integers, Ψ is R_A-*isomorphic* to R_A. *Proof:* The mapping of Ψ into R_A defined by $\mu(\psi) = \sum_a \psi(a)a^{-1}$ is obviously an additive isomorphism onto R_A. Moreover, for any $x \in A$, we have $\mu(x.\psi) = \sum_a \psi(ax)a^{-1} = x.\sum_a \psi(ax)(ax)^{-1} = x.\sum_a \psi(a)a^{-1} = x.\mu(\psi)$.

3. *Reduction to the Equivariant Case.—3.1:* The equivariant cohomology groups of an R-complex $\mathfrak{C}$ are a special case of the groups of type Ψ. The following simple but fundamental theorem states that conversely all groups of type Ψ are isomorphic to certain equivariant groups.[6]

THEOREM 1. *Let* $\mathfrak{C}$ *be an* R*-complex,* J *an Abelian group and* Ψ *a submodule of* $\Phi(R, J)$. *There is a natural isomorphism* $H_\Psi^p(\mathfrak{C}, J) \cong H^p(\mathfrak{C}, \Psi)$.

Proof: For a cochain $g^p \in C^p(\mathfrak{C}, \Psi)$ we define a cochain $Ig^p = f^p \in \check{C}^p(\mathfrak{C}, J)$ by $f^p(c_p) = g^p(c_p)(e)$ for all $c_p \in C_p$. Then $f^p(rc_p) = g^p(rc_p)(e) = (r.g^p(c_p))(e) = g^p(c_p)(r) = \psi(r)$, with $\psi \in \Psi$; hence $f^p \in C_\Psi^p(\mathfrak{C}, J)$. The mapping I is an isomorphism of $C^p(\mathfrak{C}, \Psi)$ onto $C_\Psi^p(\mathfrak{C}, J)$, which commutes with δ; it yields therefore an isomorphism of $H^p(\mathfrak{C}, \Psi)$ onto $H_\Psi^p(\mathfrak{C}, J)$.

3.2: Applying this theorem to the examples 2.3 the following results are obtained.

(a) For any R-complex $\mathfrak{C}$ and coefficient group J, $\check{H}^p(\mathfrak{C}, J) \cong H^p(\mathfrak{C}, \Phi)$, where $\Phi = \Phi(R, J)$ is the group of all additive homomorphisms of R into J.

(b) If J is an R-module, we obtain $H^p(\mathfrak{C}, J) \cong H^p(\mathfrak{C}, \Psi_0)$, which is obvious since $J \cong \Psi_0$.

(c) For a subring Q of R and a Q-module J, we have with the notations of 2.3(c), $H^p(\mathfrak{C}_Q, J) \cong H^p(\mathfrak{C}, \Psi)$, where $\Psi = Q\text{-Hom}(R, J)$.

(d') Let A be a group, B a subgroup of A, J an R_B-module, and Ψ the induced R_A-module. For an R_A-complex $\mathfrak{C}$, denoted by $\mathfrak{C}_B$ if considered as an R_B-complex, we have $H^p(\mathfrak{C}_B, J) \cong H^p(\mathfrak{C}, \Psi)$. Note that ($d'$) is a special case of ($c$).

(d'') Let A be a group, $\mathfrak{C}$ an R_A-complex, J the additive group of integers; then $H_{A\text{-fin}}^p(\mathfrak{C}, J) \cong H^p(\mathfrak{C}, R_A)$.

4. *Acyclic Complexes, Cohomology of Groups.—4.1:* An R-complex $\mathfrak{C}$ is called *free*, if for $p \geq 0$ its R-modules C_p are *free over* R. $\mathfrak{C}$ is called *acyclic* in dimension p, if $H_p = 0$. It is a well-known result[1] that if an R-complex $\mathfrak{C}$ is free and acyclic in dimensions $0 \leq p < N$, then the structure of $H^p(\mathfrak{C}, J)$, $0 \leq p < N$, is determined by R and C_{-1} (and J). Fur-

thermore, there exist for any given R and C_{-1} complexes which are free and acyclic in all dimensions; they are called "*models* for R and C_{-1}," and their groups $H^p(\mathfrak{C}, J)$, as abstract groups, the "*cohomology groups of R and C_{-1}.*"

In many cases, $H_p = 0$ for all $p \geqslant 0$ implies $\check{H}^p = 0$ for all $p > 0$ and all coefficient groups; hence, by 3.2(a), we have $H^p(\mathfrak{C}, \Phi) = 0$ for all $p > 0$, where $\Phi = \Phi(R, J)$, for any Abelian group J. If J is an R-module, it is a submodule of Φ; more precisely, J is R-isomorphic to $\Psi_0 \subset \Phi$, by the mapping q in 2.3(b).

4.2: If the ring R is the group ring R_A of an *arbitrary group* A, and C_{-1} the group of integers in which A operates trivially, then the cohomology groups of R_A and C_{-1} are called the *cohomology groups of the group* A, and are denoted by $H^p(A, J)$. Using special models,[1, 6] algebraic descriptions of various kinds of the $H^p(A, J)$ are obtained.

In this case, it is easily seen that one has for any model $\check{H}^p = 0$ for all $p > 0$; hence $H^p(A, \Phi(R_A, J)) = 0$ *for any Abelian group* J. In particular we have the result:

THEOREM 2. *Let A be a group, J an R_A-module; J can be extended to an R_A-module J' such that $H^p(A, J') = 0$ for all $p > 0$.* Namely, J' = group of all functions from A to J; an R_A-isomorphism q of J into J' is given by $q_\tau(a) = a \cdot \tau$ ($\tau \in J$, $a \in A$).[7]

This result is important in algebraic applications, and can be used in an axiomatic approach to the cohomology theory of groups.

4.3: Let B be a *subgroup* of A. We apply 3.2(d') to a model $\mathfrak{C}$ for the cohomology theory of A; since $\mathfrak{C}_B$ is a model for B, we obtain:

THEOREM 3. *Let B be a subgroup of A, J an R_B-module. The cohomology groups of B are related to those of A by the isomorphism $H^p(B, J) \cong H^p(A, \Psi)$, where Ψ is the R_A-module induced by J* (the group of all functions ψ from A to J such that $\psi(ba) = b \cdot \psi(a)$ for all $b \in B$, $a \in A$).

5. Covering Spaces.—5.1: Let $\mathfrak{C}$ be a free complex over the group ring R_A. The operations of the group A establish an equivalence relation among the chains of $\mathfrak{C}$; the equivalence classes of chains together with the induced homomorphisms ∂ constitute a new complex, denoted by $\mathfrak{C}/A$. It is easily seen that, for any coefficient group J, $\check{H}^p(\mathfrak{C}/A, J) \cong H^p(\mathfrak{C}, J)$, where, in the equivariant group $H^p(\mathfrak{C}, J)$, J is an R_A-module by trivial operations of A in J.

5.2: Now we consider a *topological space* S. By the cohomology groups $\mathfrak{H}^p(S, J)$ of S, with an Abelian group J as coefficient group, we understand the *singular groups;* i.e., the cohomology groups $\check{H}^p(\mathfrak{C}(S), J)$ of the *singular complex* $\mathfrak{C}(S)$ of S.

Assuming that S is arcwise connected, locally arcwise connected and locally simply connected, let T be its *universal covering space.* The *fundamental group* $A = \pi_1(S)$ operates in a well-known way in T; the singular

complex $\mathfrak{C}(T)$ is an R_A-complex, and $\mathfrak{C}(T)/A$ is isomorphic to $\mathfrak{C}(S)$. For an Abelian group J in which A operates trivially, we have therefore $H^p(\mathfrak{C}(T), J) \cong \check{H}^p(\mathfrak{C}(S), J)$, hence $\mathfrak{K}^p(S, J) \cong H^p(\mathfrak{C}(T), J)$. This formula remains true even if J is an arbitrary R_A-module, provided that we understand by $\mathfrak{K}^p(S, J)$ the singular cohomology groups of S *with local coefficients*,[8] corresponding to the operations of A in J.

5.3: To a subgroup B of $A = \pi_1(S)$ there corresponds a covering space $\bar{S}$ with $\pi_1(\bar{S}) = B$ (the covering being regular only if B is normal in A). T is the universal covering space not only of S, but also of $\bar{S}$. For the singular group $\mathfrak{K}^p(\bar{S}, J)$ with local coefficients from an R_B-module J, we have as before the relation $\mathfrak{K}^p(\bar{S}, J) \cong H^p(\mathfrak{C}(T)_B, J)$, where $\mathfrak{C}(T)_B$ denotes $\mathfrak{C}(T)$ considered as an R_B-complex. From $3.2(d')$ it follows that $\mathfrak{K}^p(\bar{S}, J) \cong \mathfrak{K}^p(S, \Psi)$, Ψ being the R_A-module induced by J.

THEOREM 4. *Let $\bar{S}$ be the covering space of the space S (with the topological assumptions above) corresponding to the subgroup B of $A = \pi_1(S)$. The singular cohomology groups of $\bar{S}$ and S with local coefficients are related by $\mathfrak{K}^p(\bar{S}, J) \cong \mathfrak{K}^p(S, \Psi)$, where J is an R_B-module, and Ψ the induced R_A-module* (the "induced representation of A," cf. (d') in 2.3).

5.4: If S is a polyhedron, the results of this section hold, of course, for the combinatorial cohomology groups of S, T and $\bar{S}$. This can be established directly, by applying the same arguments to the simplicial complexes associated with suitable decompositions of S, T and $\bar{S}$.

6. Transfer.—6.1: For a group A, a subgroup B of A and an R_B-module J, the "*induced R_A-module*" Ψ as defined in $2.3(d')$ has been used repeatedly in this note. In this section, some further properties of Ψ are studied under the special assumption that the index n of B in A is *finite*, and that J is an R_A-module.

As before let q be the R_A-isomorphism of J into Ψ (onto Ψ_0) defined by $q\tau(a) = a.\tau$ ($\tau \in J$, $a \in A$). A mapping t of Ψ into J is defined as follows. We take a set $X = \{x_1, \ldots, x_n\}$ of representatives of the right cosets of A modulo B and put

$$t(\psi) = \sum_{j=1}^{n} x_j^{-1}.\psi(x_j), \qquad \text{for all } \psi \in \Psi.$$

It can be verified[9] that (a) t is independent of the choice of X, (b) t is an R_A-homomorphism of Ψ into J, (c) $tq\tau = n\tau$ for all $\tau \in J$. We write M_n for the endomorphism $\tau \to n\tau$ in any additive Abelian group; hence $tq = M_n$.

For an R_A-complex $\mathfrak{C}$, we saw that $H^p(\mathfrak{C}_B, J) \cong H^p(\mathfrak{C}, \Psi)$. Therefore t induces a *homomorphism $\mathfrak{T}$ of $H^p(\mathfrak{C}_B, J)$ into $H^p(\mathfrak{C}, J)$, q a homomorphism $\mathfrak{Q}$ of $H^p(\mathfrak{C}, J)$ into $H^p(\mathfrak{C}_B, J)$ such that $\mathfrak{T}\mathfrak{Q}$ is the endomorphism M_n of $H^p(\mathfrak{C}, J)$*; $\mathfrak{T}$ is called "transfer," $\mathfrak{Q}$ "injection."

6.2: Applying this to a space S and a finite covering space $\bar{S}$ of S (cf. 5.3),

we obtain homomorphisms $\mathfrak{T}$ of $\mathfrak{K}^p(\bar{S}, J)$ into $\mathfrak{K}^p(S, J)$ and $\mathfrak{Q}$ in the opposite sense, with $\mathfrak{T}\mathfrak{Q} = M_n$ in $\mathfrak{K}^p(S, J)$. The "injection" $\mathfrak{Q}$ is easily seen to be the dual homomorphism of $\mathfrak{K}^p(S, J)$ into $\mathfrak{K}^p(\bar{S}, J)$ induced by the canonical *projection* of $\bar{S}$ onto S. The "*transfer*" $\mathfrak{T}$ *of* $\mathfrak{K}^p(\bar{S}, J)$ *into* $\mathfrak{K}^p(S, J)$ can be given by the following cochain mapping $\mathfrak{T}$: Let $\bar{f}^p$ be a singular cochain in $\bar{S}$, σ_p a singular simplex in S, and $\bar{\sigma}_p^{(j)}$, $j = 1, \ldots, n$, the n simplices in $\bar{S}$ covering σ_p; then, in the case of constant coefficients (i.e., of trivial operations of A in J), $\mathfrak{T}\bar{f}^p(\sigma_p) = \sum_j \bar{f}^p(\bar{\sigma}_p^{(j)})$, and similarly for local coefficients.

6.3: Applying 6.1 to the cohomology theory of an arbitrary *group* A and a *subgroup* B of A of finite index n (i.e., to a free acyclic R_A-complex $\mathfrak{C}$ and to $\mathfrak{C}_B$, cf. 4.2, 4.3), we obtain homomorphisms $\mathfrak{T}$ of $H^p(B, J)$ into $H^p(A, J)$ and $\mathfrak{Q}$ in the other sense, with $\mathfrak{T}\mathfrak{Q} = M_n$. A detailed discussion and application of these mappings is given in a separate paper,[9] from which we quote here only the following few results:

(*a*) If the cohomology groups of A (and B) are described[6] by means of J-valued functions of several variables in A (in B), $\mathfrak{Q}$ is given by the *restriction* of these functions from A to B.

(*b*) The transfer $\mathfrak{T}$ is a generalization of the *classical transfer*[9] $\mathfrak{V}$ of A into B/B' (B' = commutator group of B), in the sense that for trivial operations of A in J, the homomorphism of $H^1(B, J) = \mathrm{Hom}(B, J)^\epsilon$ into $H^1(A, J) = \mathrm{Hom}(A, J)$ is *dual to* $\mathfrak{V}$.

(*c*) If M_n is an automorphism of J, then Ψ is isomorphic to the direct sum $J + \Psi/J$ (where J is identified with its image in Ψ under q), hence $H^p(B, J) \cong H^p(A, J) + H^p(A, \Psi/J)$; moreover $\mathfrak{Q}$ is an isomorphism of $H^p(A, J)$ into $H^p(B, J)$.

Remark: In the case of a finite covering space $\bar{S}$ of S (6.2), one obtains similarly under the condition (*c*): $\mathfrak{K}^p(\bar{S}, J) = \mathfrak{K}^p(S, J) + \mathfrak{K}^p(S, \Psi/J)$, and $\mathfrak{Q}$ is an isomorphism of $\mathfrak{K}^p(S, J)$ into $\mathfrak{K}^p(\bar{S}, J)$. E.g., if J is the field of reals, condition (*c*) holds, and it follows that *the Betti numbers of* S *are not greater than those of* $\bar{S}$; the analog inequalities hold for the Betti numbers of a group A and those of a subgroup B with finite index.

7. Derivatives in Groups and End-points.—7.1: We conclude with a special application of our general method, in the case of the cohomology group $H^1(A, R_A)$ of a group A. It is closely related to the "derivatives in A" introduced by Fox[2] (and applied to groups given by generators and relations and to 3-dimensional topology, in particular knot theory), and on the other hand to the "end-points[10] of A."

A *derivative in* A is a function D from A to R_A satisfying $D(ab) = D(a) + a.D(b)$ for all $a, b \in A$. The condition holds for all functions of the type $D(a) = (a - e)r$, $r \in R$; these are called "trivial derivatives." Under addition, all derivatives constitute an Abelian group $\mathfrak{D}$, all trivial ones a subgroup $\mathfrak{D}_0$ of $\mathfrak{D}$. From the usual models[6] for the cohomology theory of a group A one sees immediately that $\mathfrak{D}/\mathfrak{D}_0 = H^1(A, R_A)$.

7.2: If A is finitely generated, there exists a connected finite polyhedron P with fundamental group $\pi_1(P) = A$. Let T be its universal covering space; using a suitable decomposition of T, we obtain a simplicial complex $\mathfrak{C}$ (augmented) such that (*a*) $\mathfrak{C}$ is a free R_A-complex, (*b*) $\mathfrak{C}/A$ is a *finite* simplicial complex, (*c*) $H_0(\mathfrak{C}) = H_1(\mathfrak{C}) = 0$. It follows (cf. 4.1) that $H^1(A, R_A) \cong H^1(\mathfrak{C}, R_A)$, which is isomorphic, by 3.2(d''), to $H^1_{A\text{-fin}}(\mathfrak{C}, J)$, J being the additive group of integers. Due to property (*b*) above, this group is the integer cohomology group $H^1_{\text{fin}}(\mathfrak{C}, J)$ of $\mathfrak{C}$ *based upon finite cochains*, i.e., cochains f^p with values $\neq 0$ for at most a finite number of simplices (generators of C_p). Specker proved[11] that this group is a free Abelian group of rank $e - 1$, where e is the *number of end-points* of $\mathfrak{C}$ in the sense of Freudenthal-Hopf[10] (of rank 0 if $\mathfrak{C}$ is finite). Since we have seen that $H^1_{\text{fin}}(\mathfrak{C}, J) \cong H^1(A, R_A)$, e depends only upon A. It is called the "number of end-points" of A and denoted here by $e(A)$; for a finite group one puts $e(A) = 0$.

THEOREM 5. *Let A be a finitely generated group. The group $\mathfrak{D}/\mathfrak{D}_0$ of derivatives in A modulo trivial derivatives is a free Abelian group of rank $e(A) - 1$, where $e(A)$ is the number of end-points of A (of rank 0, if A is finite).*

According to a theorem of Hopf,[10] the only possible values of $e(A)$ are $0, 1, 2, \infty$. The value of $e(A)$ is known for several examples. For a finitely generated Abelian group A, e.g., $e(A) = 2$ if the rank of A is 1, and $e(A) = 1$ if the rank is >1. *In a finite group there are only trivial derivatives;* a direct proof of this fact and of Theorem 5 (without using the general arguments of the present note and partly without assuming A to be finitely generated) will be given in a forthcoming separate paper, together with a more complete discussion.

* The results here reported were obtained in part at the Institute for Advanced Study, Princeton.

[1] Eckmann, B., PROC. NATL. ACAD. SCI., **33,** 275–281 and 372–376 (1947), where further references are given. See also Eilenberg, S., *Bull. Am. Math. Soc.*, **55,** 3–37 (1949). The present note can be considered as a continuation of the two above notes of the author; for certain details the reader is referred to them.

[2] Fox, R. H., *Bull. Am. Math. Soc.*, **55,** 276 (abstract 115) and 512 (abstract 228), 1949; see also Lyndon, R., *Ann. Math.* **52,** 650–665 (1950).

[3] The complexes considered here are "closure finite," and "augmented" (i.e., C_{-1} = group of integers, with $\partial c_0 = 1$ for the distinguished generators c_0 of C_0; H_0 is therefore the *reduced* 0-dim. homology group).

[4] By an *R-module* we understand an additive Abelian group with R as a ring of left operators (e being the unit operator); by an *R-homomorphism*, an admissible homomorphism for these operators.

[5] This result, obtained jointly by A. Wittenberg and the author, was stated in a master's thesis, Eidg. Technische Hochschule, 1949 (unpublished).

[6] Cf. Eilenberg, S., ref. 1, or Eckmann, B., *Comment. Math. Helv.*, **18,** 232–282 (1945), Chap. I and III. By Hom(A, J) we denote the group of homomorphisms of A into J.

[7] One could also take for J' an *injective* extension E of J. For any J there exists such an E, but the proof of this fact is rather complicated, and it is much simpler to take $J' = \Phi(R, J)$. It is easily seen that $\Phi(R, J)$ is injective in a *weaker* sense, which is sufficient for the cohomology theory of groups.

[8] Steenrod, N. E., *Ann. Math.*, **44**, 610–627 (1943).

[9] Eckmann, B., Cohomology of groups and transfer, to appear in *Ann. Math.*

[10] Hopf, H., *Comment. Math. Helv.*, **16**, 81–100 (1943), and Freudenthal, H., *Ibid.*, **17**, 1–38 (1944).

[11] Specker, E., *Ibid.*, **23**, 303–332 (1949), theorem IV (§5).

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*MINIMAX THEOREMS**

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Various generalizations of von Neumann's minimax theorem[1] have been given by several authors (J. Ville,[2] A. Wald,[2] S. Karlin,[2] H. Kneser,[3] K. Fan[4]). In all these theorems, the structure of linear spaces is always present. This note contains some new minimax theorems involving no linear space.

1. Let f be a real-valued function defined on the product set $X \times Y$ of two arbitrary sets X, Y (not necessarily topologized). f is said to be *convex on* X, if for any two elements $x_1, x_2 \in X$ and two numbers $\xi_1 \geqq 0$, $\xi_2 \geqq 0$ with $\xi_1 + \xi_2 = 1$, there exists an element $x_0 \in X$ such that $f(x_0, y) \leqq \xi_1 f(x_1, y) + \xi_2 f(x_2, y)$ for all $y \in Y$. Similarly f is said to be *concave on* Y, if for any two elements $y_1, y_2 \in Y$ and two numbers $\eta_1 \geqq 0$, $\eta_2 \geqq 0$ with $\eta_1 + \eta_2 = 1$, there exists an $y_0 \in Y$ such that $f(x, y_0) \geqq \eta_1 f(x, y_1) + \eta_2 f(x, y_2)$ for all $x \in X$.

THEOREM 1. *Let X, Y be two compact Hausdorff spaces and f a real-valued function defined on $X \times Y$. Suppose that, for every $y \in Y$, $f(x, y)$ is lower semi-continuous (l.s.c.) on X; and for every $x \in X$, $f(x, y)$ is upper semi-continuous (u.s.c.) on Y. Then:*

(i) *The equality*

$$\min_{x \in X} \max_{y \in Y} f(x, y) = \max_{y \in Y} \min_{x \in X} f(x, y) \tag{1}$$

holds, if and only if the following condition is satisfied: For any two finite sets $\{x_1, x_2, \ldots, x_n\} \subset X$ and $\{y_1, y_2, \ldots, y_m\} \subset Y$, there exist $x_0 \in X$ and $y_0 \in Y$ such that

$$f(x_0, y_k) \leqq f(x_i, y_0). \qquad (1 \leqq i \leqq n,\ 1 \leqq k \leqq m) \tag{2}$$

(ii) *In particular, if f is convex on X and concave on Y, then (1) holds.*

Proof: Observe first that, regardless of the condition stated in (i), the expressions on both sides of (1) are meaningful. In fact, for each $x \in X$, $f(x, y)$ is u.s.c. on the compact space Y, so that $\max_{y \in Y} f(x, y)$ exists. As maximum of a family of l.s.c. functions on X, $\max_{y \in Y} f(x, y)$ is a l.s.c. function on the compact space X and therefore $\min_{x \in X} \max_{y \in Y} f(x, y)$ exists.

(i) The necessity of the condition being trivial, we only prove its sufficiency. According to this condition,

$$\min_{x \in X} \max_{1 \leq k \leq m} f(x, y_k) \leqq \max_{y \in Y} \min_{1 \leq i \leq n} f(x_i, y)$$

holds for any two finite sets $\{x_1, x_2, \ldots, x_n\} \subset X$ and $\{y_1, y_2, \ldots, y_m\} \subset Y$. Then any real number α satisfies at least one of the two inequalities

$$\min_{x \in X} \max_{1 \leq k \leq m} f(x, y_k) \leqq \alpha, \qquad \max_{y \in Y} \min_{1 \leq i \leq n} f(x_i, y) \geqq \alpha.$$

Let $L(y; \alpha) = \{x \in X \mid f(x, y) \leqq \alpha\}$, $U(x; \alpha) = \{y \in Y \mid f(x, y) \geqq \alpha\}$, which are closed subsets of X, Y, respectively. Then for any real α and any two finite sets $\{x_1, x_2, \ldots, x_n\} \subset X$, $\{y_1, y_2, \ldots, y_m\} \subset Y$, the two intersections $\bigcap_{k=1}^{m} L(y_k; \alpha)$ and $\bigcap_{i=1}^{n} U(x_i; \alpha)$ are never both empty. As X, Y are compact, it follows that for any real α, at least one of the two intersections $\bigcap_{y \in Y} L(y; \alpha)$ and $\bigcap_{x \in X} U(x; \alpha)$ is not empty. That is, either there exists $x_0 \in X$ such that $f(x_0, y) \leqq \alpha$ for all $y \in Y$, or there exists $y_0 \in Y$ such that $f(x, y_0) \geqq \alpha$ for all $x \in X$. In other words, any real α satisfies at least one of the two inequalities

$$\min_{x \in X} \max_{y \in Y} f(x, y) \leqq \alpha, \qquad \max_{y \in Y} \min_{x \in X} f(x, y) \geqq \alpha.$$

Hence $\min_{x \in X} \max_{y \in Y} f(x, y) \leqq \max_{y \in Y} \min_{x \in X} f(x, y)$ and therefore (1).

(ii) Assume now that f is convex on X and concave on Y. In order to prove (1), it suffices to verify the condition stated in (i). Let $\{x_1, x_2, \ldots, x_n\} \subset X$ and $\{y_1, y_2, \ldots, y_m\} \subset Y$ be given. By von Neumann's minimax theorem,[1] there exist two sets $\{\xi_1, \xi_2, \ldots, \xi_n\}$, $\{\eta_1, \eta_2, \ldots, \eta_m\}$ of non-negative numbers with $\sum_{i=1}^{n} \xi_i = 1$, $\sum_{k=1}^{m} \eta_k = 1$ such that

$$\max_{1 \leq k \leq m} \sum_{i=1}^{n} \xi_i f(x_i, y_k) \leqq \min_{1 \leq i \leq n} \sum_{k=1}^{m} \eta_k f(x_i, y_k). \tag{3}$$

Since f is convex on X and concave on Y, there exist $x_0 \in X$, $y_0 \in Y$ such that

$$f(x_0, y_k) \leqq \sum_{i=1}^{n} \xi_i f(x_i, y_k), \qquad (1 \leq k \leq m) \tag{4}$$

$$f(x_i, y_0) \geqq \sum_{k=1}^{m} \eta_k f(x_i, y_k). \qquad (1 \leqq i \leqq n) \tag{5}$$

Then (2) follows from (3), (4), (5).

In (ii) of Theorem 1, we have an easily applicable sufficient condition for equality (1). It can be used to provide simple proofs for minimax theorems for infinite games (for instance, the generalized Ville's minimax theorem as stated in our earlier note[4] is a special case of part (ii) of Theorem 1). Since the proof of (ii) is based on von Neumann's minimax theorem, its application in proving a minimax theorem for infinite games amounts essentially to a reduction of the latter to von Neumann's theorem for finite games.

2. Theorem 2 below generalizes Kneser's minimax theorem[3] by eliminating the structure of linear spaces. Theorem 2 also improves (ii) of Theorem 1. Our proof of Theorem 2 is a modification of Kneser's proof of his theorem.

THEOREM 2. *Let X be a compact Hausdorff space and Y an arbitrary set (not topologized). Let f be a real-valued function on $X \times Y$ such that, for every $y \in Y$, $f(x, y)$ is l.s.c. on X. If f is convex on X and concave on Y, then*

$$\min_{x \in X} \sup_{y \in Y} f(x, y) = \sup_{y \in Y} \min_{x \in X} f(x, y). \tag{6}$$

Proof: Observe first that the expressions on both sides of (6) have meaning, although their values may be $+\infty$. We divide the proof of (6) into four steps:

(i) *Let $y_0 \in Y$ be such that $X_0 = \{x \in X | f(x, y_0) \leqq 0\}$ is not empty. If we replace X by X_0, and restrict f on $X_0 \times Y$, then the hypothesis of Theorem 2 remains fulfilled.*

We need only to verify that f restricted on $X_0 \times Y$ is convex on X_0. Let $x_1, x_2 \in X_0$, and $\xi_1, \xi_2 \geqq 0$, $\xi_1 + \xi_2 = 1$ be given. By convexity of f on X, there exists $x_0 \in X$ such that

$$f(x_0, y) \leqq \xi_1 f(x_1, y) + \xi_2 f(x_2, y) \text{ for all } y \in Y. \tag{7}$$

Since $f(x_1, y_0) \leqq 0$, $f(x_2, y_0) \leqq 0$, the case $y = y_0$ of (7) implies $f(x_0, y_0) \leqq 0$, i.e., $x_0 \in X_0$.

(ii) *If $\{y_1, y_2\} \subset Y$ is such that*

$$\max_{k=1,2} f(x, y_k) > 0 \text{ for all } x \in X, \tag{8}$$

then there exists $y_0 \in Y$ such that

$$f(x, y_0) > 0 \text{ for all } x \in X \tag{9}$$

Let $X_k = \{x \in X | f(x, y_k) \leqq 0\}$, $(k = 1, 2)$, which are disjoint closed sets in X. We assume that none of them is empty (otherwise (ii) is trivial).

We have $-f(x, y_1) \geqq 0$ and $f(x, y_2) > 0$ for $x \in X_1$, so that $\dfrac{-f(x, y_1)}{f(x, y_2)}$ is u.s.c. and $\geqq 0$ on X_1. Let $x_1 \in X_1$ and $\mu_1 \geqq 0$ be such that

$$\max_{x \in X_1} \frac{-f(x, y_1)}{f(x, y_2)} = \frac{-f(x_1, y_1)}{f(x_1, y_2)} = \mu_1. \tag{10}$$

Similarly, let $x_2 \in X_2$ and $\mu_2 \geqq 0$ be such that

$$\max_{x \in X_2} \frac{-f(x, y_2)}{f(x, y_1)} = \frac{-f(x_2, y_2)}{f(x_2, y_1)} = \mu_2. \tag{11}$$

We claim that $\mu_1\mu_2 < 1$. To verify this, we may assume $\mu_1\mu_2 \neq 0$. Since $f(x_1, y_1) \leqq 0$, $f(x_2, y_1) > 0$, we can find $\xi_1, \xi_2 \geqq 0$ such that $\xi_1 + \xi_2 = 1$ and

$$\xi_1 f(x_1, y_1) + \xi_2 f(x_2, y_1) = 0. \tag{12}$$

f being convex on X, there exists $x_0 \in X$ such that

$$f(x_0, y) \leqq \xi_1 f(x_1, y) + \xi_2 f(x_2, y) \text{ for all } y \in Y. \tag{13}$$

From (12), (13), we have $f(x_0, y_1) \leqq 0$ and therefore, by (8), $f(x_0, y_2) > 0$, so that

$$0 < \xi_1 f(x_1, y_2) + \xi_2 f(x_2, y_2).$$

Using (10), (11) and the fact $\mu_1 > 0$, the last inequality may be written

$$\xi_1 f(x_1, y_1) + \mu_1\mu_2\xi_2 f(x_2, y_1) < 0,$$

which compared with (12) yields $\mu_1\mu_2 < 1$.

Take ν_1, ν_2 such that $\nu_1 > \mu_1$, $\nu_2 > \mu_2$, $\nu_1\nu_2 = 1$. Let

$$\eta_1 = \frac{1}{1 + \nu_1} = \frac{\nu_2}{1 + \nu_2}, \qquad \eta_2 = \frac{\nu_1}{1 + \nu_1} = \frac{1}{1 + \nu_2}.$$

Then

$$\eta_1 f(x, y_1) + \eta_2 f(x, y_2) > 0 \text{ for all } x \in X. \tag{14}$$

In fact, if x is not in $X_1 \cup X_2$, (14) is trivial. If $x \in X_1$, we have $0 \leqq f(x, y_1) + \mu_1 f(x, y_2) < f(x, y_1) + \nu_1 f(x, y_2) = (1 + \nu_1)[\eta_1 f(x, y_1) + \eta_2 f(x, y_2)]$. Similarly one verifies (14) for $x \in X_2$. Finally the existence of $y_0 \in Y$ with property (9) follows from (14) and the concavity of f on Y.

(iii) *If a finite set* $\{y_1, y_2, \ldots, y_m\} \subset Y$ *is such that*

$$\max_{1 \leq k \leq m} f(x, y_k) > 0 \text{ for all } x \in X, \tag{15}$$

then there exists $y_0 \in Y$ *satisfying* (9).

We prove this by induction on m. Let $X_m = \{x \in X \mid f(x, y_m) \leqq 0\}$.

We assume that X_m is not empty (otherwise we take $y_0 = y_m$). By (i), we can apply our induction-assumption to f restricted on $X_m \times Y$. Since $\max_{1 \leq k \leq m-1} f(x, y_k) > 0$ for all $x \in X_m$, there exists $y_{m+1} \in Y$ such that $f(x, y_{m+1}) > 0$ for all $x \in X_m$. Then $\max_{k=m,\, m+1} f(x, y_k) > 0$ for all $x \in X$. By (ii), there exists $y_0 \in Y$ satisfying (9).

(iv) *For any real number α, either there exists an $x_0 \in X$ such that $f(x_0, y) \leqq \alpha$ for all $y \in Y$, or there exists an $y_0 \in Y$ such that $f(x, y_0) > \alpha$ for all $x \in X$.* (Therefore the right side of (6) is not less than the left side, and consequently the two are equal.)

Suppose the first alternative is not true. Then $\bigcap_{y \in Y} L(y; \alpha)$ is empty, where $L(y; \alpha) = \{x \in X | f(x, y) \leqq \alpha\}$. As X is compact, there exists a finite set $\{y_1, y_2, \ldots, y_m\} \subset Y$ such that $\bigcap_{k=1}^{m} L(y_k; \alpha)$ is empty. That is, $\max_{1 \leq k \leq m} f(x, y_k) > \alpha$ for all $x \in X$. Then an application of (iii) to the function $f - \alpha$ shows that the second alternative is true.

3. The next theorem is free of topological structures. This is made possible by generalizing von Neumann's almost periodic functions on a group.[5] A real-valued function f defined on the product set $X \times Y$ of two arbitrary sets X, Y (not topologized) is said to be *right almost periodic*, if f is bounded on $X \times Y$ and if, for any $\epsilon > 0$, there exists a finite covering $Y = \bigcup_{k=1}^{m} Y_k$ of Y such that $|f(x, y') - f(x, y'')| < \epsilon$ for all $x \in X$, whenever y', y'' belong to the same Y_k. *Left almost periodic* functions are defined similarly. However, every right almost periodic function on $X \times Y$ is also left almost periodic and vice versa.[6] Thus we simply use the term *almost periodic*.

THEOREM 3.[7] *Let f be a real-valued almost periodic function on the product set $X \times Y$ of two arbitrary sets X, Y (not topologized). Then:*

(i) *The equality*

$$\inf_{x \in X} \sup_{y \in Y} f(x, y) = \sup_{y \in Y} \inf_{x \in X} f(x, y) \tag{16}$$

holds, if and only if the following condition is satisfied: For any $\epsilon > 0$, any two finite sets $\{x_1, x_2, \ldots, x_n\} \subset X$ and $\{y_1, y_2, \ldots, y_m\} \subset Y$, there exist $x_0 \in X$, $y_0 \in Y$ such that

$$f(x_0, y_k) - f(x_i, y_0) \leqq \epsilon. \quad (1 \leqq i \leqq n,\ 1 \leqq k \leqq m). \tag{17}$$

(ii) *In particular, if f is convex on X and concave on Y, then* (16) *holds.*

Proof: We only prove the sufficiency part of (i). Consider an arbitrary $\epsilon > 0$. Let $X = \bigcup_{i=1}^{n} X_i$ be a finite covering of X with the property

corresponding to ϵ required in the definition of left almost periodicity. Let $Y = \bigcup_{k=1}^{m} Y_k$ be a finite covering of Y with the property corresponding to ϵ required in the definition of right almost periodicity. Let $x_i \in X_i$ $(1 \leqq i \leqq n)$, $y_k \in Y_k$ $(1 \leqq k \leqq m)$. Then $\sup_{y \in Y} f(x, y) \leqq \max_{1 \leq k \leq m} f(x, y_k) + \epsilon$ holds for all $x \in X$; and $\inf_{x \in X} f(x, y) \geqq \min_{1 \leq i \leq n} f(x_i, y) - \epsilon$ holds for $y \in Y$.[8] Hence

$$\inf_{x \in X} \sup_{y \in Y} f(x, y) \leqq \inf_{x \in X} \max_{1 \leq k \leq m} f(x, y_k) + \epsilon, \tag{18}$$

$$\sup_{y \in Y} \inf_{x \in X} f(x, y) \geqq \sup_{y \in Y} \min_{1 \leq i \leq n} f(x_i, y) - \epsilon. \tag{19}$$

Using our condition, there exist $x_0 \in X$, $y_0 \in Y$ satisfying (17). From (17), (18), (19), we get

$$0 \leqq \inf_{x \in X} \sup_{y \in Y} f(x, y) - \sup_{y \in Y} \inf_{x \in X} f(x, y) \leqq 3\epsilon,$$

which holds for any $\epsilon > 0$. Thus (16) is proved.

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[1] Von Neumann, J., "Zur Theorie der Gesellschaftsspiele," *Math. Annalen*, **100**, 295–320 (1928); von Neumann, J., and Morgenstern, O., *Theory of Games and Economic Behavior*, Princeton University Press, Princeton, 1944, pp. 153–155.

[2] For the references concerning minimax theorems of J. Ville, A. Wald, and S. Karlin, see the Bibliography in *Contributions to the Theory of Games*, edited by H. W. Kuhn and A. W. Tucker, Princeton University Press, Princeton, 1950.

[3] Kneser, H., "Sur un théorème fondamental de la théorie des jeux," *C. R. Acad. Sci. Paris*, **234**, 2418–2420 (1952).

[4] Fan, K., "Fixed-Point and Minimax Theorems in Locally Convex Topological Linear Spaces," *Proc. Nat. Acad. Sci.*, **38**, 121–126 (1952).

[5] Von Neumann, J., "Almost periodic functions in a group, I," *Trans. Am. Math. Soc.*, **36**, 445–492 (1934); Maak, W., "Eine neue Definition der fast-periodischen Funktionen," *Abhandl. Math. Sem. Hans. Univ.*, **11**, 240–244 (1936).

[6] Let f be right almost periodic. Given $\epsilon > 0$, let $Y = \bigcup_{k=1}^{m} Y_k$ be a finite covering of Y with the property corresponding to $\epsilon/3$ required in the definition of right almost periodicity. Let $y_k \in Y_k$ $(1 \leqq k \leqq m)$. For each k, $f(x, y_k)$ is a bounded function on X, there is a finite covering $X = \bigcup_{i=1}^{n_k} X_i^{(k)}$ of X such that $|f(x', y_k) - f(x'', y_k)| < \frac{\epsilon}{3}$ whenever $x', x'' \in X_i^{(k)}$ for some i. Then the common refinement of these m finite coverings of X has the property corresponding to ϵ required in the definition of left almost periodicity.

[7] It should be said that the absence of topological structures in Theorem 3 is more apparent than real. In fact, the almost periodicity of f is a necessary and sufficient condition in order that X, Y can be made into two precompact (in the sense of Bourbaki, but not necessarily separated) uniform spaces in such a way that f is uniformly continuous on the product uniform space $X \times Y$.

[8] Here we see that the hypothesis in Theorem 3 on almost periodicity of f can be considerably weakened.

ON THE POINCARÉ POLYNOMIALS OF THE CLASSICAL GROUPS—ADDENDUM

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In this ADDENDUM to our note with the above title in these PROCEEDINGS[1] we wish to amplify a detail in our deduction of the Poincaré polynomial $P_n(z) = (1 + z)(1 + z^3) \ldots (1 + z^{2n-1})$ of the n-dimensional linear group in order to show more clearly the connection between $P_n(z)$ and Euler's infinite product $\Pi(z) = (1 + z)(1 + z^3) \ldots$. This connection is as follows: The coefficient of z^k, $k = 1, 2, \ldots$, in $\Pi(z)$, being the number of partitions of k into unequal odd parts, is the number of self-associated partitions of k; on the other hand, the coefficient of z^k in $P_n(z)$ is the number of self-associated partitions of k which do not contain more than n elements. For example, the coefficient of z^8 in $P_n(z)$ is 2 if $n \geqslant 4$, since 8 has the two self-associated partitions (421^2) and (3^22) neither of which contains more than 4 elements, while the coefficient of z^8 in $P_3(z)$ is 1, the self-associated partition (421^2) being forbidden. The coefficients of z^8 in $P_2(z)$ and $P_1(z)$ are zero, these polynomials being of degrees 4 and 1, respectively, and, generally, all self-associated partitions of $k > n^2$ contain more than n parts. Thus we have the following convenient characterization of the kth Betti number, B_k, of the n-dimensional linear group: B_k is the number of self-associated partitions of k which do not contain more than n parts. This characterization is equivalent to that given by Weyl.[2]

In our previous note we showed that $B_k = B_{n^2-k}$ is the number of times that $\sum_{(\alpha)} \{\alpha\}(s^{-1})\{\alpha^*\}(s)$ contains the identity representation of the n-dimensional linear group, the summation being over all partitions (α) of k. Since $\{\alpha\}$ vanishes if it contains more than n parts we may confine our attention to those partitions of k which do not contain more than n parts. If (λ) and (μ) are any two partitions of k we denote by $\Gamma_{(\lambda)}$ and $\Gamma_{(\mu)}$, respectively, the corresponding irreducible representations of the symmetric group on k letters and observe that $\{\lambda\}(s^{-1})\{\mu\}(s)$ contains the identity representation of the linear group exactly as often as $\Gamma_{(\lambda)} \times \Gamma_{(\mu)}$ contains the identity representation of the symmetric group, this number being 1 if $(\mu) = (\lambda)$ and 0 if $(\mu) \neq (\lambda)$. Thus $\{\alpha\}(s^{-1})\{\alpha^*\}(s)$ contains the identity representation of the linear group once if $(\alpha^*) = (\alpha)$ and does not contain it if $(\alpha^*) \neq (\alpha)$. Hence B_k is the number of self-associated partitions of k which do not contain more than n parts.

[1] Murnaghan, F. D., these PROCEEDINGS, **38**, 608–611 (1952).

[2] Weyl, H., *The Classical Groups*, P. 237, Theorem (7.11.B), Princeton University Press, Princeton, N. J. (1939).

ON EIGENFUNCTION EXPANSIONS

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Given a Hilbert space $\mathfrak{H}$ over the complex numbers and in $\mathfrak{H}$ a spectral family $P(E)$. By the latter is meant as usual a function $P(E)$ whose argument varies over all elements E of some σ-field $\mathfrak{E}$ of subsets of some space $\mathfrak{Y}$ and whose value is for each $E \in \mathfrak{E}$ a projection $P(E)$ of $\mathfrak{H}$ such that the mapping $E \to P(E)$ is a σ-Boolean algebra homomorphism. We assume that the elements of $\mathfrak{H}$ are square integrable functions or equivalence classes of square integrable functions on some measure space $\mathfrak{X}$ such that the square norm $\|f\|^2$ in $\mathfrak{H}$ is given by the square integral over $\mathfrak{X}$. Then the problem of eigenfunction expansion may be formulated as follows: Do there exist functions $s_r(x, y)$ defined on $\mathfrak{X} \times \mathfrak{Y}$ and measures $m_r(E)$ defined on $\mathfrak{E}$ such that if one puts for every $f(x) \in \mathfrak{H}$

$$\hat{f}_r(y) = \int_{\mathfrak{X}} s_r(x, y) f(x)\, dx \tag{1}$$

then it should follow that $\hat{f}_r(y)$ be m_r-measurable and square-integrable and that

$$\int_{\mathfrak{X}} |f(x)|^2\, dx = \sum_r \int_{\mathfrak{Y}} |\hat{f}_r(y)|^2\, dm_r(y). \tag{2}$$

Here the functions $s_r(x, y)$ must be such that the integral in (1) exists for every $f(x) \in \mathfrak{H}$, at least in the sense of limit in the mean, and $s_r(x, y)$ should for each fixed r and y be an eigenfunction of the family $P(E)$ in some sense to be specified.

The second half of the problem is that of the existence of an inversion formula, i.e., whether the functions $s_r(x, y)$ are such that

$$\int_{\mathfrak{Y}} \sum_r s_r(x, y) \hat{f}_r(y)\, dm_r(y). \tag{3}$$

exists in some sense such that as a function of x it is an element of $\mathfrak{H}$ and that

$$f(x) = \int_{\mathfrak{Y}} \sum_r s_r(x, y) \hat{f}_r(y)\, dm_r(y) \tag{3a}$$

where the equality shall mean equality of elements of $\mathfrak{H}$ [for example, almost everywhere equality of functions in case $\mathfrak{H} = \mathfrak{L}_2(\mathfrak{X})$].

Let $g_r(x)$ be an arbitrary element of $\mathfrak{H}$ and $\mathfrak{H}_r$ the smallest closed linear subspace of $\mathfrak{H}$ which contains all elements of the form Bg_r, where B varies over all polynomials in the operators $P(E)$. For every $E \in \mathfrak{E}$ and $g \in \mathfrak{H}$ put

$$m(E) = m_g(E) = \langle P(E)g, g \rangle, \tag{4}$$

in particular we write

$$m_r(E) = \langle P(E)g_r, g_r \rangle$$

where $\langle , \rangle$ denotes the inner product in $\mathfrak{H}$. If f_r is an arbitrary element of $\mathfrak{H}_r$, then there exists an m_r-measureable function $\hat{f}_r(y)$ such that

$$f_r = \int_{\mathfrak{Y}} \hat{f}_r(y) d[P(y)g_r] \tag{5}$$

holds in the sense of the operational calculus for Hilbert space, so that the integral in (5) exists in the strong topology of $\mathfrak{H}$. Moreover by the spectral theory for abstract Hilbert spaces the function $\hat{f}_r(y)$ is square integrable with respect to m_r and determined by f_r and g_r uniquely up to subsets of $\mathfrak{Y}$ of m_r-measure zero such that

$$\|f_r\|^2 = \int_{\mathfrak{Y}} |\hat{f}_r(y)|^2 \, dm_r(y). \tag{6}$$

If, now, f is an arbitrary element of $\mathfrak{H}$, form first the above subspace $\mathfrak{H}_1$ for some $g_1 \in \mathfrak{H}$, then with some element g_2 contained in the orthogonal complement of $\mathfrak{H}_1$ form the closed linear subspace $\mathfrak{H}_2$; continuing in this way one obtains pairwise orthogonal subspaces $\mathfrak{H}_r$ which span $\mathfrak{H}$. If f_r now denotes the orthogonal projection of f into $\mathfrak{H}_r$ and $\hat{f}_r(y)$ the function corresponding to f_r under the correspondence (5) then equation (6) implies an equation of the form (2) for every element $f(x)$ of $\mathfrak{H}$. Thus part (1), (2) of the problem of eigenfunction expansions would be solved if it can be shown that the correspondence $f(x) \leftrightarrow \hat{f}_r(y)$ defined by (5) is actually given by a system of integral equations of the form (1).

For this purpose put

$$g(x, E) = (P(E)g)(x) \qquad \text{or} \qquad g(E) = P(E)g \tag{7}$$

for any element $g = g(x)$ of $\mathfrak{H}$. Then (5) can be rewritten as

$$f_r(x) = \int_{\mathfrak{Y}} \hat{f}_r(y) \, dg_r(x, y) \tag{8}$$

where the equality sign and the meaning of the integral in (8) must of course be taken in the same sense as in (5).

Thus the problem arises when the set function $g(x, E)$ is for a given x absolutely continuous with respect to the measure $m(E) = \langle P(E)g, g \rangle$. If E_j are elements of $\mathfrak{E}$ for which $m(E_j) \to 0$ then we have, of course

$$\lim_j \langle P(E_j)g, g \rangle = \lim_j \|P(E_j)g\|^2 = 0. \tag{9}$$

Now if $\mathfrak{H}$ is the space $\mathfrak{L}_2(\mathfrak{X})$ of all equivalence classes of complex valued

measurable square integrable functions on $\mathfrak{X}$ then we may conclude from (9) merely that for almost every x a subsequence of $g(x, E_j)$ tends to zero, but one cannot conclude absolute continuity of $g(x, E)$ in E for any one x!

If, on the other hand, we take for $\mathfrak{H}$ a function space in which strong convergence in $\mathfrak{H}$ implies pointwise convergence of the functions for every $x \in \mathfrak{X}$, then we obtain immediately the desired absolute continuity and hence the existence of the Radon-Nikodym derivative

$$\frac{\partial g(x, E)}{\partial m(E)} = s(x, y) \text{ for } x \in \mathfrak{X} \text{ and } y \in \mathfrak{Y}, \tag{10}$$

and therefore we may conclude that (5) or (8) implies (1) in this case. Here one has to assume, of course, that the Radon-Nikodym theorem holds. This will certainly be true in the case of a separable space. So we see for instance that the above proves the existence of an eigenfunction expansion of the type (1), (2) for such spaces $\mathfrak{H}$ as the space of all square integrable complex holomorphic functions with respect to a suitable measure, for any spectral family $P(E)$ whatsoever.

If, however, $\mathfrak{H}$ is the whole space $\mathfrak{L}_2(\mathfrak{X})$ on some measure space $\mathfrak{X}$ then restrictions on the family $P(E)$ are necessary in general. Let $A = \int y dP(y)$ be a self-adjoint (hypermaximal) operator defined on some dense linear subset $\mathfrak{D}$ of $\mathfrak{H}$ and assume that there exists a complex number c for which the operator $K = (A - cI)^{-1}$ is a bounded everywhere defined integral operator of the Carleman type; i.e., we assume that there exists a function $k(x, \xi)$ defined (and measurable) on $\mathfrak{X} \times \mathfrak{X}$ which is square integrable in ξ for almost every x and such that

$$\{(A - cI)^{-1}f\}(x) = (Kf)(x) = \int_{\mathfrak{X}} k(x, \xi)f(\xi)\, d\xi \tag{11}$$

for every $f \in \mathfrak{L}_2(\mathfrak{X})$. Since A is self-adjoint $\mathfrak{Y}$ can be taken to be the real line and $m(E)$ a Lebesgue-Stieltjes measure. Since $g(E) = P(E)g$ is clearly in the domain $\mathfrak{D}$ of A whenever $E \in \mathfrak{E}$ is a bounded subset of the real line we may put[1]

$$g^*(E) = (A - cI)g(E) \tag{12}$$

for every $g \in \mathfrak{H}$ and bounded $E \in \mathfrak{E}$; then

$$g(E) = Kg^*(E), \text{ i.e., } g(x, E) = \int k(x, \xi)g^*(\xi, E)\, d\xi \tag{13}$$

for every $E \in \mathfrak{E}$. Using now the assumption that $k(x, \xi)$ is square integrable in ξ for fixed x we can write (13) as an inner product in $\mathfrak{H}$

$$g(x, E) = \langle g^*(E), k_x \rangle \text{ for almost every } x, \tag{14}$$

where k_x denotes for fixed x the function $\bar{k}(x, \xi)$ considered as an element of $\mathfrak{H} = \mathfrak{L}_2(\mathfrak{X})$. Hence

$$|g(x, E)| \leq \|g^*(E)\| . \|k_x\| \tag{15}$$

holds for every fixed $E \in \mathfrak{E}$ for all x outside some subset $\mathfrak{X}_{0,E}$ of $\mathfrak{X}$ of measure zero, which may, of course, depend on E. However, for each fixed E the function $g(x, E)$ may be changed arbitrarily on a subset of $\mathfrak{X}$ of measure zero. Hence we may put

$$g_0(x, E) = \begin{cases} g(x, E) & \text{for } x \tilde{\in} \mathfrak{X}_{0,E} \\ 0 & \text{for } x \in \mathfrak{X}_{0,E} \end{cases} . \tag{16}$$

Then we obtain from (15)

$$|g_0(x, E)| \leq \|g^*(E)\| . \|k_x\| \text{ for every } x \in \mathfrak{X} \text{ and } E \in \mathfrak{E}. \tag{17}$$

Hence using (12) and (7) we get

$$|g_0(x, E)| \leq \| (A - cI)P(E)g\| . \|k_x\| \text{ for every } x \in \mathfrak{X}. \tag{18}$$

Since $m(E)$ is now a Lebesgue-Stieltjes measure, it is clear that if E_j are sets $\in \mathfrak{E}$ for which $m(E_j) \to 0$ we may take them to be uniformly bounded subsets of the real line. Then we have

$$\|AP(E_j)g\|^2 = \int_{E_j} |y|^2 d\|P(y)g\|^2 = \int_{E_j} |y|^2 \, dm(y) \leq \gamma . m(E_j)$$

where γ is a constant independent of j. Hence it follows that

$$\lim m(E_j) = 0 \text{ implies } \lim g_0(x, E_j) = 0 \text{ for every fixed } x \in \mathfrak{X}. \tag{19}$$

This proves that given any element $g(x)$ of $\mathfrak{L}_2(\mathfrak{X})$, then $g_0(x, E)$ as defined by (7) and (16) is for every fixed $x \in \mathfrak{X}$ an absolutely continuous set-function of E with respect to the measure $m(E) = < P(E)g, g >$, provided that there exists an integral operator of the form (10) which is of the Carleman type. Hence, applying the Radon-Nikodym theorem to the Lebesgue-Stieltjes measure $m_g(E)$ we obtain for each $g(x) \in \mathfrak{L}_2(\mathfrak{X})$ a function $s(x, y)$ by substituting $g_0(x, E)$ in (10). Thus, in particular, if we do this for a suitable family of functions $g_r(x)$ for which the above spaces $\mathfrak{H}_r$ are pairwise orthogonal and span $\mathfrak{H}$ we obtain functions $s_r(x, y)$ for which (1) and (2) hold. *This proves the existence of an eigenfunction expansion of the form (1), (2) for any self-adjoint hypermaximal operator* $A = \int y dP(y)$ *for which there exists an operator (11) of the Carleman type.*

This can be applied to the classical problem of eigenfunction expansions for differential operators. Let then $\mathfrak{L}_2(\mathfrak{X})$ be the Lebesgue space over a real (finite or infinite) interval $\mathfrak{X}$ and L a formally self-adjoint (system of) ordinary differential operator(s). Under very general conditions on L the existence of a hypermaximal operator A is known, such that the intersection of the domains of L and A is dense in $\mathfrak{L}_2(\mathfrak{X})$ and such that there exists a Green's function $k(x, \xi)$ satisfying (11). Thus the existence of formulas (1) and (2) follows in this case at once from the above with hardly

any computation; also knowledge of the Green's function $k(x, \xi)$ for only one suitable complex number c is seen to be sufficient for this purpose. In particular it is seen that the above can be applied to avoid the use of the integro-differential equation[2] which H. Weyl employed in his derivation of a Plancherel formula for second-order ordinary differential equation. Also H. Weyl's subdivision into Grenzpunkt and Grenzkreisfall can be restricted to the existence proof of Green's function $k(x, \xi)$ which we need only for one complex number c. The same remarks apply and hence lead to a simplification of the treatment of Eigenfunction expansion for ordinary differential operators given by K. Kodaira. Again the above implies that the integro-differential equation[3] can be avoided and the derivation of the analog of equations (1), (2) and (3a) simplified.

It should be noted that whenever the functions $s_r(x, y)$ defined by equation (10) above are for fixed r product measurable on $\mathfrak{X} \times \mathfrak{Y}$ so that the Fubini-theorem holds, then a standard argument shows that (3a) follows from (1) and (2). In the particular case of differential operators these conditions on $s_r(x, y)$ are satisfied. Moreover in this case of a differential operator L (satisfying the above-mentioned condition) a simple standard argument shows that $s_r(x, y)$ is for fixed r and y an eigenfunction of L in the ordinary sense (whereas for more general operators $s_r(x, y)$ can be interpreted as functions corresponding to eigendifferentials).

We conclude by remarking that there is a large class of partial differential operators for which there exists a Green's kernel $k(x, \xi)$(cf. (11) above) such that our method applies. However the extent of this class of partial differential operators does not seem to be known.

The above is also related to the generalized Peter-Weyl-Plancherel-formula on certain locally compact topological groups. This will be discussed elsewhere.

[1] Compare the following argument with the proof of theorem 5.4 given by K. Kodaira in "On Ordinary Differential Equations of Any Even Order and the Corresponding Eigenfunction Expansion," *Am. J. Math.*, *LXXII*, 502–544 (1950).

[2] Equation (55), *Math. Ann.*, **68**, 239 (1910).

[3] Equation (5.33) on p. 531 of K. Kodaira, *loc. cit.*

*ABNORMALITY OF DOWN PIGMENTATION ASSOCIATED WITH EXPERIMENTALLY PRODUCED SKELETAL DEFECTS OF CHICKS**

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Communicated by L. C. Dunn, November 5, 1952

We have previously reported that teratological syndromes involving beak and posterior extremities can be produced in chicken embryos or newly hatched chicks by injecting at an appropriate developmental stage insulin,[1] boric acid[2] or pilocarpine hydrochloride[3] into the yolk sac of eggs. All three substances, if they are injected at 96 hours of incubation, call forth such defects; insulin treatment continues to lead to similar symptoms during the fifth day of development, but our present report is

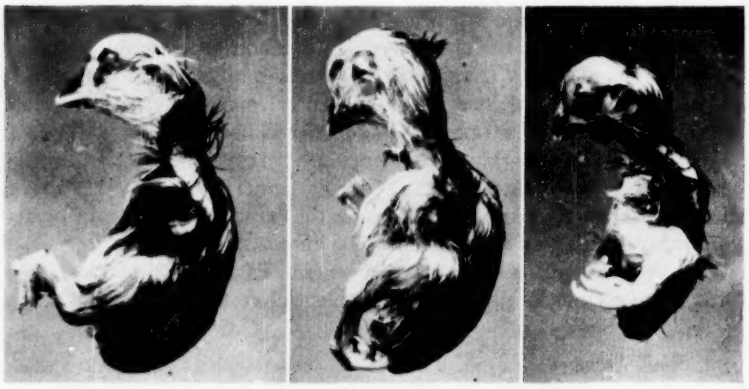

FIGURE 1

Black Minorca embryos after treatment with 2 units insulin at 96 (*left*) and 120 hrs. (*center and right*) of incubation.

essentially based on experiments performed at the 96-hour stage. Although there is great similarity in the target area in which insulin, boric acid and pilocarpine engender deviations from normal development, their teratogenic action shows considerable specificity in detail. Briefly summarized, the principal differences in teratological end-effects due to the three compounds are as follows: in the facial skeleton, insulin treatment is typically responsible for a shortened maxilla or a parrot-beak condition; pilocarpine most commonly leads to a shortening of the mandible, at times associated with facial clefts; and boric acid tends to produce ex-

treme reductions in mandible size, coupled with cleft palate and facial coloboma. In the posterior extremities the characteristic effect of insulin is a micromelia, in which all long bones are reduced in length, but with little damage to the toes; pilocarpine is responsible for syndactylism and (after higher doses) shortening and bending of tarsometatarsus, as well as reduction in size (or even suppression) of the first toe; boric acid affects the tarsometatarsus in a way similar to that of pilocarpine, but the fourth toe is preferentially reduced or suppressed. For details the reader must be referred to the earlier reports.

These experiments had been performed with eggs of White Leghorn fowl. Similar tests have now been made with eggs of Black Minorca fowl, and in these we found that, in addition to the skeletal malformations,

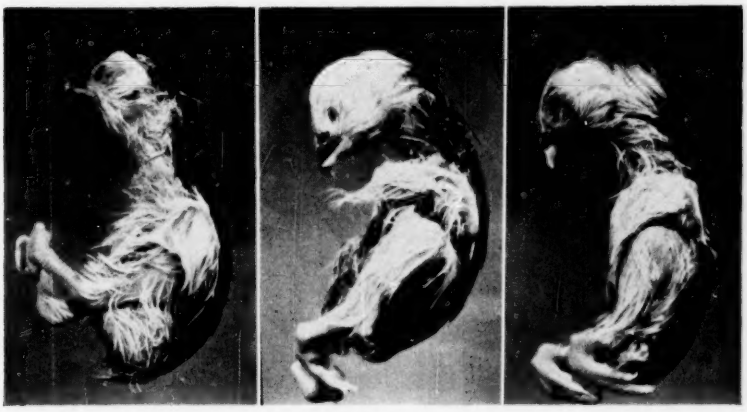

FIGURE 2

Black Minorca embryos after treatment with 2.5 mg. boric acid at 96 hrs. of incubation.

insulin, boric acid and pilocarpine also produce changes in down pigmentation. Before we report on these changes, a brief description must be given of the pattern of normal down coloration. Prior to and at the time of hatching, the down of Black Minorca chicks is solidly black with the following exceptions: the ventral body surface is predominantly covered with white down; throat and abdomen are always white, but in the pectoral region there is frequently an intermixture of black and white, resulting in a bluish appearance, or a small black area may be present, separating throat and abdomen. A few small white marks are often found in the face. These consist of a narrow band of white down just above the eyes; at times a small white spot is found between eyes and ears, and often a small triangular area of white down extends from the base of the beak

toward the throat. The alula region of the wing generally has a few white down plumules. Otherwise, the dorsal and lateral surface of the body and the legs are covered with black down.

Insulin, boric acid and pilocarpine, injected at 96 hours of development and, therefore, at a time when they produce the malformations which have been described at the beginning of this report, tend to modify the pattern of down pigmentation of Black Minorca chicks. The effects which the three compounds have on pigmentation are so similar that they can be discussed together.

In chicks which had hatched after treatment with one of the teratogenic

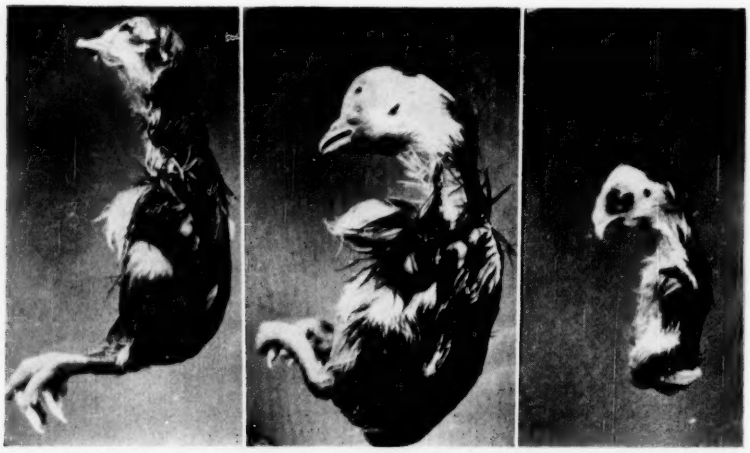

FIGURE 3

Black Minorca embryos after treatment with 6 mg. pilocarpine hydrochloride at 96 hrs. of incubation. The specimen on the left has an almost normal skeleton (legs slightly shortened) and the least reduction in black pigmentation of all embryos illustrated.

substances, slightly more white down was frequently found in the face and in the wing tips than occurs normally. The band of white down on the ventral surface was never interrupted in the thoracic region, as it frequently is in normal Black Minorca chicks. The great majority of these chicks were morphologically normal, only a few showing minor skeletal defects. In fully developed and morphologically normal chicks which had failed to hatch, the changes in pigmentation were similarly slight. In chicks of treated origin non-pigmented feathers persisted in the first definitive plumage, but disappeared in subsequent molts, the adult plumage becoming entirely black.

In chicks which had died during the last four days of incubation and

which showed skeletal defects, the changes of pigmentation were more common and often more extensive (Figs. 1–3). The failure of melanin formation always occurred as a broadening of the white ventral area and a more or less extensive lack of pigmentation in the terminal body regions (head, wings, legs). The dorsum invariably retained black down. There was a clear relation between extent of skeletal abnormality and degree of restriction in black pigmentation. When the skeletal abnormality was slight (e.g., Fig. 3, left), the abnormal lack of black down was usually confined to parts of the face, the distal portion of the wings and the scales of the toes. With higher degrees of skeletal defects, the failure of melanin formation was likely to include the whole head and neck, all of the wings, and large areas of the legs, including the scales of the tarsometatarsus. The correlation between degree of skeletal malformation and extent of restriction in black down pigmentation was by no means perfect, but—especially if the hatched chicks and the fully developed and morphologically normal embryos are included in the comparison—the interrelationship was sufficiently clear to warrant the assumption of common epigenetic pathways. This conclusion is reinforced by the fact that the injection of amounts of teratogenic substances which are insufficient to bring about skeletal malformations or the treatment in (somewhat earlier or somewhat later) stages during which no skeletal defects can be produced, also are ineffective as far as changes of down pigmentation are concerned. Furthermore, embryos and chicks which, due to administration of protective supplements, remain free of skeletal defects after treatment with insulin, boric acid or pilocarpine, also developed normal pigmentation.

The most effective supplementary treatment in order to reduce or prevent the teratological effects of insulin, boric acid and pilocarpine is the simultaneous injection of nicotinamide. The protective action of nicotinamide was first discovered with reference to skeletal abnormalities [3–6] and has now been found to hold equally in regard to the disturbances of pigmentation. The effectiveness of nicotinamide in preventing or at least in reducing the incidence and severity of malformations after the injection of insulin, boric acid or pilocarpine at 96 hours of development was the most important single evidence in a series of facts which led us to the conclusion that these skeletal defects arise on the basis of disturbances of carbohydrate utilization. The present finding that in connection which all three teratogenic substances nicotinamide supplementation produces the same beneficial and protective results as far as down pigmentation is concerned, can hardly be considered a matter of coincidence. It must, therefore, be concluded that the disturbances of pigmentation and the skeletal defects found in our material trace back to an interference with metabolic peculiarities which melanoblasts and certain skeletal primordia have in common. The pattern in which melanin formation was suppressed in the down of treated

chicks indicates either that melanoblast activity was interfered with in the affected regions or that pigment cells on their way from the neural crest were damaged and hindered in their migration, thereby preventing invasion of and pigment formation in the down of the more distal body parts. A combination of these mechanisms may, of course, have been operative.

Presumably the most important part of our observations on the consequences of treating 96-hour chicken embryos with insulin, boric acid or pilocarpine is the unquestionable existence of a correlation between the degree of skeletal deformity and the extent of disturbance in pigmentation. These observations seem to justify the hypothesis, to be tested by future experiments, that damage to neural crest derivatives may be the causative factor in the origin of the whole syndrome of skeletal and pigmentary abnormalities.

Summary.—Teratogenic substances (insulin, boric acid, pilocarpine hydrochloride) which, when injected into the yolk sac of developing chicken eggs at 96 hours of incubation, produced a syndrome of skeletal malformations involving beak and posterior extremities, also led in embryos with black down to a restriction of melanin formation. In Black Minorca embryos this suppression of black pigmentation occurred as a widening of the area which normally remains unpigmented. Supplementary nicotinamide served to reduce or prevent in the skeleton as well as in down pigmentation the damage produced by the teratogenic compounds. The metabolic disturbances leading to the damage are, therefore, presumably the same in both instances. There was a definite correlation between degree of skeletal abnormality and extent of restriction in black pigmentation. This fact suggests the hypothesis that damage to neural crest derivatives may be responsible for the whole syndrome.

* Supported by a grant from the American Cancer Society, on recommendation of the Committee on Growth, National Research Council.

[1] Landauer, W., *J. Expt. Zool.*, **105**, 145–172 (1947).

[2] Landauer, W., *Ibid.*, **120**, 469–508 (1952).

[3] Landauer, W., *Ibid.* (in print).

[4] Landauer, W., *Ibid.*, **109**, 283–290 (1948).

[5] Landauer, W., and Rhodes, M. B., *Ibid.*, **119**, 221–261 (1952).

[6] Zwilling, E., *Ibid.*, **109**, 197–214 (1948); *Arch. Biochem.*, **33**, 228–242 (1951).

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